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Alyami, Mohammed Saeed S

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By

Mohammed Saeed Salem Alyami

Student Number: 500326334

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Supervisors: Dr Vera Fitzsimmons-Thoss
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Abstract

Petroleum, also known as “crude oil”, may enter the environment in large and small volumes due to accidental spillages such as acts of war, or terrorism or through natural processes. Crude oil is comprised of a complex mixture of compounds of which the main constituents are aliphatic hydrocarbons, with smaller proportions of aromatic compounds. The aims of this research are is to assess the background concentrations of total petroleum hydrocarbons (TPHs) in the Saudi Arabian desert, assess their ecotoxicology and to develop simple means of remediation.

In this project, physio-chemical properties for Saudi Arabian desert sands (n=16) were determined. The pH ranged from 8.06 to 9.64. The sand moisture ranged from 0.04% to 0.68%. The organic matter content for sand samples ranged from 0.22% to 0.91%. The percentage of Carbon in sand ranged from 0.05% to 3.30%, while Nitrogen ranged from 0.01% to 0.03%. Elemental analysis was applied to all sand samples and Calcium (ranging from 1310 mg/kg to 14823 mg/kg) was found to be the most abundant element with an average of 5543 mg/kg, followed by Iron (ranging from 134 mg/kg to 6839 mg/kg) with an average of 2590 mg/kg. In addition, Aluminium ranged from 1065 mg/kg to 4575 mg/kg with an average of 1963 mg/kg. Magnesium ranged from 166 mg/kg to 2007 mg/kg with an average of 987 mg/kg.

Hydrocarbon fractionation of crude oil and diesel into aliphatic and aromatic fractions was successfully done using column chromatography. For diesel, around 80% to 90% of the TPH were recovered using fractionation. For crude oil, approximately 30% of TPH were recovered. Therefore, around 20% of diesel and 70% of crude oil was lost because some compounds were volatile or stuck to the silica gel.

Deionized water was also used to extract hydrocarbons from five of the sixteen samples. The water extractable hydrocarbons ranged from C_{21} to C_{36} with a TPH concentration of 586 mg/kg in sample Riyadh and 6651 mg/kg in Hufuf. Individual hydrocarbon concentrations also differed. The concentration of C_{36} in sample Hufuf was 3.48 mg/kg and C_{29} concentration was 1260 mg/kg. Dichloromethane (DCM) was similarly used to extract hydrocarbons from 13 samples. The concentration of TPHs extracted ranged from 234 mg/kg in sample Khobar to 34708 mg/kg in Karj. Hydrocarbons ranged from C_{20} to C_{31} with the same distribution in all samples. The C_{24} and C_{25} had the highest concentrations, while C_{31} was mostly the lowest. The concentrations in sample Hufuf were 137.93 mg/kg for C_{21} and 3142.43 mg/kg for C_{24}.

To assess the ecotoxicology of the background hydrocarbons in crude oil contaminated sand (0.5% up to 10%) and control sand (0 % of TPHs), the germination of seven different types of plant seeds (ryegrass, wheat, coriander, lettuce, radish, cabbage red drumhead and cabbage greyhound) was studied noting germination rate, root and shoot length. Variation was observed in respect to the concentration of crude oil and the type of seeds. Germination experiments were conducted in both the United Kingdom (UK) and the Kingdom of Saudi Arabia (KSA) in order to determine the effect of temperature on plant growth and percentage seed germination. For some plants, seed germination was better in the UK than in KSA. Conversely, some plants germinated better in KSA than in the UK. In the UK, wheat roots and shoots grew better in contaminated sand at all contamination levels than in the control sand (there was a significant difference between roots and shoots compared to control sand as p value < 0.05, apart from shoot at 0.5 % and 5 %, as there was no significant difference as p value > 0.05). In KSA, wheat root and shoot growth decreased in contaminated sand at all contamination levels, except for a 0.5% contamination. In the UK, coriander root and shoot growth decreased at all contamination levels, while the root and shoot growth increased at all contamination levels in the KSA.

Wheat and ryegrass had longer root and shoot length in crude oil contaminated sand, up to 5%, compared to the control. Hence, these species could play a role in phytoremediation for hydrocarbon contaminated environments. For samples grown in the UK, crude oil contamination had no detrimental effect on root and shoot length for lettuce and wheat (up to 10% contamination) and rye grass (up to 2% contamination). For samples grown in KSA, crude oil contamination had no detrimental effect on root length for lettuce and coriander (up to 10% contamination), rye grass (up to 2% contamination) and radish and wheat (up to 0.5% contamination). Seed germination rate varied widely (5% up to 100%) among the control sand and Saudi sands.

The ecotoxicology study showed that although existing petroleum hydrocarbon contamination is potentially detrimental to the environment, it can be assessed using seed germination assays as long as the chosen seed is appropriate for the climate. Lettuce seeds are recommended.

To study remediation, sodium dodecyl sulfate (SDS) and consumer goods, such as washing up liquid, shower gel and shampoo, were assessed for their ability to remediate hydrocarbon contamination from sand. SDS solution (30 ml of 0.1% w/v), and different shaking times were investigated. Shaking for 60 minutes at 350 rpm, was more effective shaking for 20 and 40 minutes. Higher levels of crude oil contamination produced higher levels of hydrocarbon remediation. At 60 minutes SDS (0.1%) removed 10.6%, 18% and 22% of TPH at a level of 2, 5 and 10% of crude oil contamination, respectively. Next, SDS was replaced with consumer goods to illustrate the potential for simple remediation using resources locally available such as washing up liquid, shower gel and shampoo. It was found that washing up liquid removed more of the contamination than shampoo and shower gel. At 60 minutes, washing up liquid removed 15%, 17% and 20.5% of crude oil contamination at 2, 5 and 10% of crude oil, respectively.

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I. List of Abbreviations

Abbreviation	Meaning
CMC	Critical Micelle Concentration
HLB	Hydrophilic Lipophilic Balance
O/W	Oil / Water
W/O	Water/Oil
API	American Petroleum Institute
TPHs	Total Petroleum Hydrocarbons
TAPs	Total Aliphatic Hydrocarbons
PAHs	Polycyclic Aromatic Hydrocarbons
DOR	Dispersant oil ratio
USEPA	United State Environmental Protection Agency
NAPLs	Non Aqueous Phase Liquid
SDS	Sodium Dodecyl Sulfate
KSA	Kingdom of Saudi Arabia
UK	United Kingdom
NAPLs	Non-Aqueous Phase Liquid
ICP-AES	Inductively Coupled Plasma Emission Spectroscopy
GC-MS	Gas Chromatography–Mass Spectrometry
CS	Control Sand

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1 Chapter 1: Hydrocarbons and Their Remediation in Desert Environments

1.1 Introduction.

The kingdom of Saudi Arabia is situated in the southwest Asia (in the Middle East), which is considered the bulk of the Arabian Peninsula. The estimated land mass of Saudi Arabia is approximately 2,217,949 square kilometres but only around 1% of the total district is suitable for agriculture. However, Saudi Arabia is one of the largest producers of crude oil in the world and this has played a significant role in the economic development of the Arabian Peninsula and countries adjacent to the Persian Gulf (Silva, Rita de Cássia FS, Almeida *et al.,* 2014).

The Petroleum industry has also played a significant role in the world economy and society. It serves as one of the main sources of energy in most developed counties. The demand fro crude oil around the world is projected to increase by approximately 37% between 2006 to 2020. Many of the products used by humans are made from petroleum-based compounds such as gasoline, kerosene, and diesel fuel oil. It is also the precursor for polymers. Petroleum is a complex mixture of hydrocarbons, aromatics, sulphur, nitrogen, oxygen and metals such as chromium, copper, iron, manganese, nickel, vanadium and zinc (Adebiyi *et al.*, 2016). The main constituents are aliphatic and aromatic compounds, resins and asphaltenes. A major concern is the detrimental effect that these compounds pose to humans and the ecosystem during the various stages of production, processing, storage and transport.

Petroleum hydrocarbons have been either intentionally or accidently released into the environment in large and small volumes by humans (Singh and Subhash, 2014), but they can also be released during natural disasters. Accidental oil pollution of the environment occurs during oil transportation, oil storage and the process of drilling (Urum *et al.,* 2006 and Khamehchiyan, Hossein and Tajik, 2007). Due to the high demand for oil and its derivatives, crude oil obtained from land and sea drilling needs to be transported and stored. Transportation from the oil fields to the consumer involves as many as 15 to 20 transfers, from oil tankers to pipelines to tank trucks. In Saudi Arabia, spillage occurs on a large scale when there are major transportation accidents, pipeline bursts or mechanical failure of pumps (Dibble and Bartha, 1979a; Widrig and Manning, 1995; Carman *et al.*, 1998).

An example of the intentional release of crude oil into the environment is the Gulf war. In 1991, the Iraq invasion of Kuwait left one of the worst environmental disasters of war. It has been estimated that approximately 798 oil wells were ignited, 149 damaged and 45 oil well left over-flowing. The gushing oil wells spread and covered the Kuwait desert and reached the Saudi Arabian desert (Almutairi, 2017) (PEC, 1999) (Khordagui, 1993). The extent of marine and atmospheric pollution resulting from the Gulf war warrants the assessment of background concentrations. Readman *et al.* in 1992 and 1996, reported that oil contamination of the marine environment reached the coastline of Saudi Arabia, 400 km from where it had been released. The estimated spilled oil ranged from 0.5 million to 8 million barrels (Readman *et al.*, 1996 and Readman *et al.*, 1992) and was estimated to take a year for the marine environment to recover.

Atmospheric pollution from burning crude oil wells in Kuwait, resulted in an average concentration of polycyclic aromatic hydrocarbons of 22 $\mu g\ m^{-3}$ (Husain and Aramco, 1998) and 23 $\mu g\ m^{-3}$ for benzo[a]pyrene. The non-methane hydrocarbon concentration in the Mansoria area of Kuwait City increased to 352% from 1990 to 1991 and the methane concentration increased by 50% from 1990 to 1991. Similarly the Rega area had an increase in non-methane hydrocarbon of 48 % from 1990 to 1991, while the methane decreased by 4% from 1990 to 1991 (Husain and Aramco, 1998). Furthermore, the smoke density and particulate matter in the air has been reported to decrease air temperature.

Land is the most prone to petroleum hydrocarbon pollution and is of global interest because it ruins the chemical balance of the ecosystem, causes growing economic loss, loss of fertility, reduction in productivity and the environment takes many years to recover (Khordagui, 1993).

Plants are directly and indirectly impacted by hydrocarbon contamination due to the effect on water quality and pollution of the soil, by making toxic minerals accessible in the rhizosphere. Hydrocarbon pollution causes loss of organic matter content (OMC), deterioration of soil structure and mineral loss (K^+, Na^+, SO_4^{2-}, PO_4^{3-}, NO_3^-) (Baruah, Deka and Baruah, 2016). Hydrocarbon contaminated soil also affects earthworms by increasing their mortality (Wong Chai *et al.* 1999).

Due to the negative effect of soil pollution (in the case of Saudi Arabia sandy soil) on human health and the ecosystem, contaminated soil needs to be remediated. To minimize the harmful effects of soil contamination, different types of remediation strategies have been employed such as soil washing, bioremediation, phytoremediation, electro kinetic remediation and thermal desorption. Among these, soil washing has attracted attention globally because it is considered a more time-efficient method compared to bioremediation and phytoremediation and because it is largely unaffected by climatic factors (Urum, Pekdemir *et al*,. 2003, Urum, Pekdemir *et al*,. 2004). Soil washing is a mechanical process that utilizes aqueous solutions to chemically treat and get rid of large volumes of contaminants from soil. The addition of additives, such as surfactants, helps in the desorption and solubilisation of petroleum hydrocarbons from the soil.

Surfactants are a class of amphiphilic compounds, which simultaneously possess both hydrophobic and hydrophilic groups in their structure. This unique physicochemical property is what enables surfactants to increase the water solubility of petroleum hydrocarbons from sand, thereby promoting the bioremediation of the pollutants.

The majority of remediation research for hydrocarbon pollution has been conducted on soil (Wang *et al.*, 2019) (Mussa and Sharaa, 2014) (Cunningham *et al.*, 1997) (Płaza *et al.*, 2005) (Semer and Reddy, 1996) (Kostecki and etal, 1999) (Urum *et al.*, 2006) (Cunningham *et al.*, 1996) (Khan, Troquet and Vachelard, 2005) yet land cover on the Arabian Peninsula and neighbouring Gulf countries is comprised of large areas of desert. Deserts are sandy environments and contrary to soil, has little organic matter, low water content and substantially fever plants or other organisms. The Rub Al-khali of the Kingdom of Saudi Arabia (KSA) is also known as the empty quarter and is also described as the largest sandy desert in the world (Groucutt, White *et al*,. 2015), and an area of considerable potential for both oil and gas reserves. (**Figure 1**).

Figure 1 Empty Quarter (Rub Al-khali) (Groucutt, White *et al.*, 2015).

Because few studies have assessed hydrocarbon pollution, ecotoxicity or remediation for sand (Arelli *et al.*, 2018) (Khamehchiyan, Hossein and Tajik, 2007), the focus here is the assessment of background hydrocarbon concentration, composition, ecotoxicity and remediation of sand containing hydrocarbons.

1.2 Sand.

Sand is largely composed of inorganic components, derived from a combination of primary and secondary minerals and rocks (Sparks, 2003). Primary minerals can be described as chemically unaltered deposits resulting from crystallization of molten lava and includes quartz and feldspar. Secondary minerals are formed from the weathering of primary minerals and includes aluminosilicate minerals such as kaolinite $Al_2Si_2O_5(OH)_4$, montmorillonite, M_x (Al, Mg, $Fe^{2+})_4Si_8O_2O(OH)$ (M = Na and Ca), and aluminium oxides such as gibbsite, $[Al(OH)_3]$. The mineral composition of sand can vary greatly of which silicon dioxide (SiO_2), usually in the form of quartz, is a major component. The pore diameter between sand particles is usually large allowing for easy water drainage (Sparks, 2003).

1.3 Crude Oil.

Crude oil also known as “Petroleum”, is made up of a considerable number of components from light gases to heavy materials, which boil below ambient temperature and above 550 °C, respectively. Crude oil deposits contain a complex mixture of hydrocarbons and other compounds that can be in form of solids (bitumen, tars), liquids and gases (natural gases), extracted from the topmost layer of the Earth and can range from C_1 - C_{60}. According to American Petroleum Institute (API), crude oil can also contain different inorganic elements such as H (14%), C (84%), S from 1% - 3%, N (1%) and O (1 - 0.1%). Depending on the source of the crude oil, both the properties and characteristics can differ such as the viscosity, density, and oil type. Based on its characteristics, crude oil can be categorized as heavy or light, sweet or sour, and paraffinic or naphthenic (Klimisch, H. J., *et al* 2002, Xueqing Z hu., *et al* 2001).

Crude oil can be described as heavy or light based on viscosity (oils resistance to flow), density and gravity. The viscosity of crude oil is defined as the oil’s resistance to flow and shear. At higher temperatures, the oil is less viscous. The temperature at which liquid crude oil becomes semi-solid (more viscous), is called the pour point. The viscosity of crude oil is affected by its composition. Variation in temperature and evaporation rate, can affect also the density and viscosity of oils, while the interfacial tension of the oil does not appear to have any correlation with crude oil viscosity (Okieimen, Okieimen, 2002).

Gravity also defines an oil as heavy or light. API gravity can be calculated by the following formula:

$$\text{API gravity} = \frac{141.5}{\text{specific gravity}} - 131.5$$

Crude oil with an API gravity of more than 31.1 is known as light oil. Light oil is characterized to be light in colour with low metal and sulphur content. Crude oil with an API gravity of less than 22.3, is known as heavy oil. Heavy oil is characterized by being dark in colour and having high metal and sulphur content. A considerable amount of asphaltenes and aromatic fractions can be found in heavy oil (Klimisch, H. J. *et al.,* 2002).

Sweet oil is crude oil which contains a minor amount of carbon dioxide (CO_2) and sulphur. However, crude oil that has a major amount of CO_2 and sulphur is known as sour oil (high impurity and toxicity). Furthermore, crude oil contains which predominantly contains paraffinic groups are is referred to as paraffinic oil and when crude oil contains mostly naphthenic and aromatic hydrocarbons, it is referred to as Naphthenic (Klimisch, H. J. *et al.,* 2002).

1.4 Hydrocarbons.

1.4.1 Hydrocarbons in crude oil.

Hydrocarbons are organic compounds, which are composed solely of carbon and hydrogen atoms. (**Figure 2**) (Silva, Rita de Cássia FS, Almeida *et al.,* 2014). Chemicals such as hexane, benzene, toluene, naphthalene are common examples of compounds that occur in crude oil and are constituents of several petroleum products. The hydrocarbons found in crude oil are divided into three main classes: aliphatic (paraffins), asphaltenes, and aromatics.

- *Aliphatic*: Alkane hydrocarbons ranging between C_1 to approximately C_{40} and sometimes even up to C_{60}. These can further be divided into a) straight chain alkanes, also called n-alkanes; b) branched alkanes, also called iso-alkanes and c) cycloalkanes, also called naphthenes. Aliphatic hydrocarbons constitute the greatest fraction of crude oil (Balba, Al-Awadhi *et al.,* 1998).

- *Aromatics*: these compounds are composed of at least one benzoid ring and are classified into monocyclic aromatic and polycyclic aromatic hydrocarbon compounds (PAHs). The monocyclic aromatic compounds have one benzene ring such as benzene, toluene, xylene, etc., PAHs compounds consist of two rings or more benzoid rings such as anthracene, phenanthrene. The rings can be fused such as naphthalene or linked such as biphenyl (Xueqing Z hu, *et al.*, 2001).

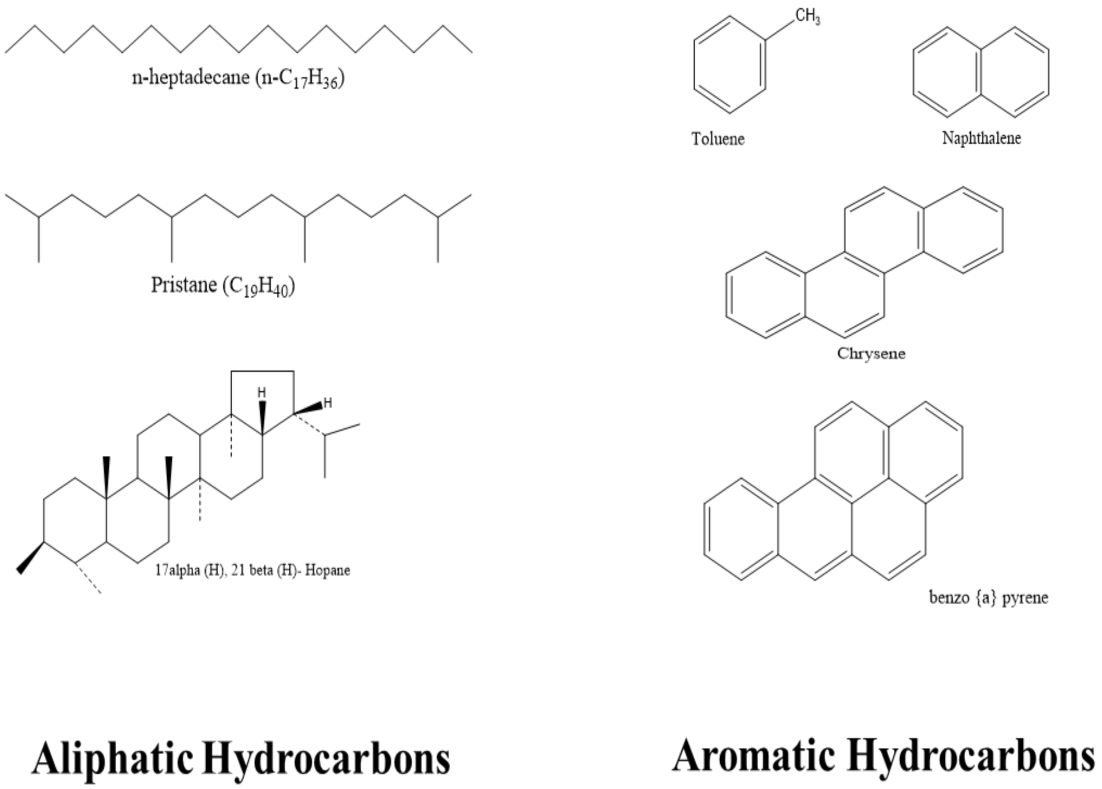


Figure 2 Chemical structure of some hydrocarbons found in crude oil.

1.4.2 Hydrocarbons in Diesel Fuel.

In the research conducted here, diesel fuel is an example of a crude oil product which is comprised mostly of long chain aliphatic hydrocarbons ranging between 9 to 28 carbon atoms. Diesel fuel is distilled at temperatures of 160°C - 390°C, yielding more than 200 compounds, each with its own physical and chemical properties. The exact mixture of hydrocarbons in diesel fuel is affected by factors such as the source of crude oil, refinery process and nature of oil shipment (Scheepers, Bos, 1992) and hence the exact characteristic of diesel fuel varies.

Diesel fuel also contains a small percentage of PAHs and sulphur (0.1 – 0.5 wt%) (Scheepers, Bos, 1992). The colour of diesel fuel varies from colourless to brown and is partially soluble in water (5 mg L^{-1} at 20°C) and Log K_{ow} 3.3 – 7.06 (ATSDR, 1995).

1.4.3 Hydrocarbon release into the Environment.

Crude oil found naturally in the soil (in the case of Saudi Arabia sand) may not necessarily be considered a contaminant, but it can become a problem, if it migrates towards the groundwater level. It only takes 1 g of oil to contaminate 1 m^3 of groundwater to 1 ppm. Therefore, if the crude oil is disturbed from its natural source or is spilled accidentally as a result of transportation and storage, it can be considered a pollutant, because it could impact negatively on the surrounding environment.

Anthropogenic activities such as crude oil production, storage, extraction, drilling, exploration, leakage during refining and transportation result in hydrocarbon release. Contamination of land by petroleum hydrocarbons may also come from railway yards and transportation refuelling areas where continual small spills have led to adverse oil pollution. In addition, a lot of industries, factories and army bases have their own fuel storage tanks for refuelling vehicles, which are probable sources of oil contamination. In addition, the large number of petrol stations around the country may add to contamination of the environment through refuelling accidents and storage related issues (Bossert and Bartha, 1985; Ururahy *et al.,* 1999; Brown *et al.,* 1998; Jergensen *et al.*, 2000).

Many oil spill accidents have been reported over the world (**Table 1**) and recent estimates suggest that the greatest source of petroleum hydrocarbon pollution worldwide is crude oil (57.2%) followed by diesel fuel oil (32.1%), petrol or gasoline (8.3%), other oils such as lubricating oil, used motor oil, and mineral oil (1.4%), and finally jet fuel (< 0.01%) (Singh, Srivastava *et al.,* 2008).

Table 1 Accidental Hydrocarbons spillage into the environment.

Spillage Year	Spillage Location	Amount Spilled	Cause to the Environment	Reference
2011, November	Sedco 706 oil rig in Brazil	5943 L of oil	Spilled oil covering approximately 163 km^2 causing considerable damages to the environment	(Silva, Rita de Cássia FS, Almeida et al. 2014), (Souza et al., 2014)
2010, July	Dalian, China	1500 tons of oil	Caused an environmental contamination along the coast of Dalian	(Liu et al., 2013)
2010, April	Gulf of Mexico (Deepwater Horizon)	3 to 4 million barrels of oil	Considered as the largest environmental disaster to the marine environment in the world	(Sammarco et al., 2013)
1991, January	Gulf War (Kuwait War)	8 to 11 million barrels of crude oil	Crude oil contamination covered approximately 400 km^2 from spill point to Saudi coastline causing serious damages to the environment	(Husain and Aramco, 1998) (Readman et al., 1996) (Freije and Freije, 2018)
1979, June	Gulf of Mexico, Ixtoc 1 oil well	140 million gallons of oil	Caused environment problems	(Aransas and Aransas, 1980)
1967, April	off the Scilly Isles, in the UK	25 - 36 million gallons of oil	Caused considerable damages to the environment with about 30000 of sea birds and aqueous animals killed	(Viggi, Presta et al. 2015) (Dunnet et al., 1987)

1.4.4 Environmental Fate of Hydrocarbons.

1.4.4.1 Hydrocarbons in Water.

After a crude oil spill in a marine a slick layer of oil is formed on the water. There are eight main weathering processes that determine the fate of the hydrocarbons in a marine environment and these include spreading, evaporation, emulsification, dispersion and dissolution (early stage of a spill) as well as sedimentation, photo oxidation and biodegradation (late stage of a spill).

Wind, waves, and water turbulence contribute to breaking up the oil slick and spreading the oil over a considerable area of the water surface. Oil viscosity, and specific gravity, in addition to temperature, wind speed, and water intensity can affect the spreading of oil (Xueqing Z hu, *et al.*, 2001).

Spreading of the oil on the water surface leads to processes such as evaporation and dissolution by increasing the surface area of the oil (**Figure 3,** Reed, Johansen *et al.*, 1999). Evaporation reduces the volume of an oil spill, but in turn increases the viscosity and density of the remaining fractions. It has been reported, that approximately 95% of benzene from an oil spill evaporated while less than 5% remained in the water. The National Research Council (NRC) in 2005 reported that evaporation of spilled oil accounts for 20% - 50% of environmental contamination depending on spilled oil type together with wind speed, water temperature, waves and water turbulence (NRC 2005).

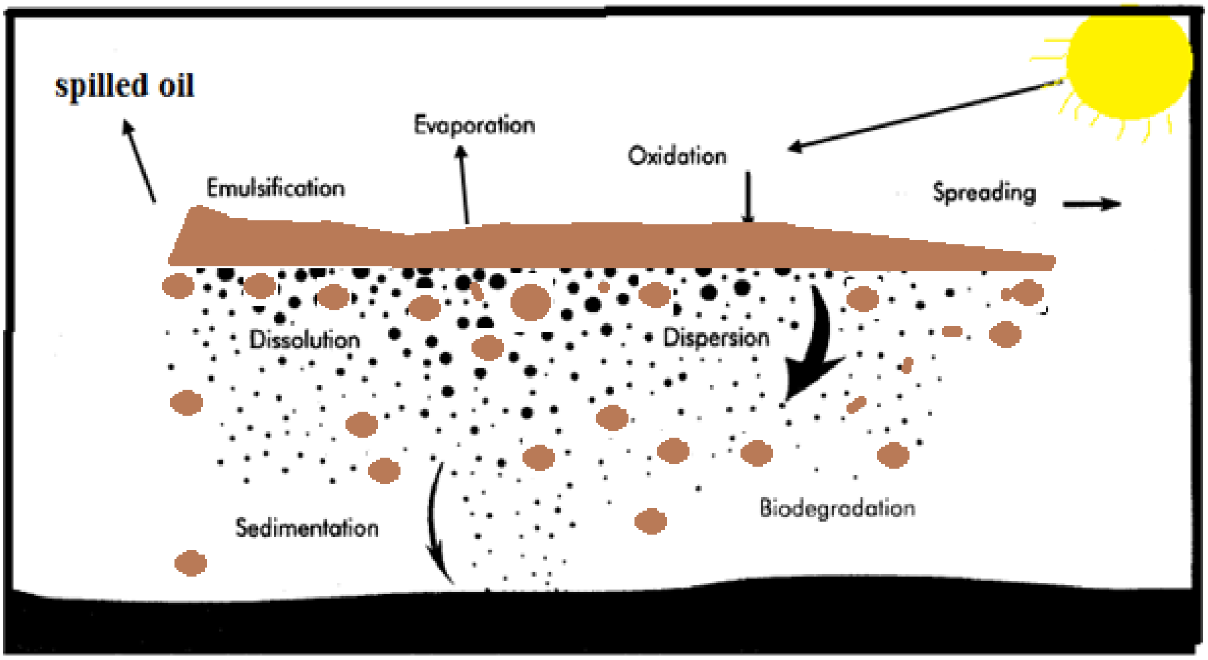


Figure 3 Weathering processes affecting oil spill in a water environment (adapted from Zhu *et al.*, 2001).

Crude oil is a highly hydrophobic material with most of its constituents having very low water solubility and water often becomes trapped as small droplets within the spilled oil. This forms a water-in-oil emulsion which increases weathering and degradation times, prolonging the water contamination.

To the contrary, dissolution of the soluble hydrocarbons such as benzene and toluene increases biodegradation but dissolution can also increase the availability of toxic compounds such as xylene (BTX) (ITOPF, International Tanker Owners Pollution Federation Limited, 2002).

1.4.4.2 Hydrocarbons on Land.

In general, the effect of an oil spill on sand depends to some extent on factors such as sand/ type, geology, temperature, and size of the oil pool. Once released, petroleum hydrocarbons tend to bind and interact with soil particles, specifically the mineral fractions, through a combination of physical and chemical mechanisms, such as sorption, complexation and precipitation (Megharaj *et al.*, 2011). The contamination of soil with petroleum hydrocarbons also affects the aeration because of the displacement of air.

The behaviour of crude oil can change when mixed with sand. The rate of movement of crude oil as well as the degree of sand penetration can be affected by crude oil viscosity for example light oil and its products such as diesel and gasoline have a low specific gravity and quickly penetrate sand. Simultaneously, the evaporation of the light oil is quite rapid (can evaporate at room temperature) and they tend not to linger in the sand, which might be an advantage for the environment (Obe, 2001) (Adebiyi *et al.*, 2016).

Heavy oil has a high density (specific gravity) and is considered more toxic than light oil to the environment because it remains longer on the surface of the sand (USDA 1998, Maletić, Dalmacija *et al.* 2013). Heavy oil also contains compounds with over 60 C atoms with high boiling points and which are not easily biodegraded.

Aromatic compounds, such as polycyclic aromatic hydrocarbons (PAHs), mostly interact with organic matter through mechanisms such as physical and chemical adsorption, solubilisation, hydrolysis, and photosensitization, affecting their mobility and bioavailability throughout the sand (Šašek et al., 2003; Steinberg, 2009).

It has been found that sand generally contains between 0.001 and 300,000 mg kg^{-1} total PAHs depending on the source of contamination (Bamforth, Singleton 2005) (Kanaly and Harayama, 2000). Wilcke (2007) reported that based on the global pattern of PAHs in sand (0.004-186 mg kg^{-1}), countries in Central Europe such as Germany and Czech Republic contain more PAHs than China, Russia, Thailand, America, Brazil and Ghana. However, a more recent study by Loganathan and Lam (2011), stated that there were more PAHs in Indian sands compared to Africa, Iran, Brazil, Russia, Canada and Australian sands. Thus, PAHs could be classified as a pollutant with a high degree of variation in their concentration levels in different regions. Malawska and Wiołkomirski (2001) reported that the degree of sand pollution depends on the concentration of PAHs (**Table 2**).

Table 2 Standard Polycyclic aromatic hydrocarbon concentration in sand.

PAHs* Concentration in Sand mg/kg	**Sand Regarded**
Less than 0.6	Unpolluted
0.6 to 1	Slightly polluted
1 to 5	Polluted
5 to 10	Heavy polluted
More than 10	Extremely polluted

*Polycyclic Aromatic Hydrocarbons.

1.4.5 Impact of Hydrocarbon Pollution.

Petroleum hydrocarbon spills create a serious threat to the wellbeing of both the aquatic and terrestrial environments as well as human health (Wong, Chai *et al.*, 1999; van Gestel, van der Waarde, Jaap J. *et al.*, 2001; Viggi, Presta *et al.*, 2015; Dibble and Bartha, 1979b). Petroleum hydrocarbons destabilize ecosystem functions, such as photosynthesis, respiration and the nitrogen (N) cycle (Schafer *et al*., 2009).

In order to assess the impact of petroleum hydrocarbons (PHCs) on human and environmental health associated with polluted land, petroleum hydrocarbons have been subdivided into specified ranges of equivalent carbon chain lengths or fractions. These fractions are further subdivided according to their physio-chemical properties and toxicological characteristics (CCME, 2008). For example, **Table 3** shows the Canadian national soil standards and guidelines which characterize PHC fractions under different risk management schemes, land use pattern and physical conditions of the soil. In these guidelines, petroleum hydrocarbons have been divided into four fractions. Fraction 1 (F_1: C_6-$C_{10)}$ represents the volatile fraction, Fraction 2 (F2: C10-C16) represents the semi- volatile fraction. Fraction 3 (F_3: C_{16}-C_{34}) represents the non-volatile hydrocarbons and Fraction 4 (F_4: C_{34} – C_{50}^{+}) encompasses compounds with the lowest volatility and solubility (CCME, 2008; ATSDR, 2011b).

Table 3 Example of Canada national soil quality guidelines for PHC fractions for surface sand.

Land Use Pattern	**Soil Type**	**F1 (C6- C10)**	**F2 (C10 – C16)**	**F3 (C16 – C34)**	**F4 (C34 - C50+)**
		mg kg^{-1}			
Residential Road side / forest	Coarse-grained	30[a]	150	300	2800
	Fine- grained	210 (170[b])	150	1300	5600
Agricultural	Coarse-grained	30[a]	150	300	2800
	Fine- grained	210 (170[b])	150	1300	5600
Commercial	Coarse-grained	320 (240[b])	260	1700	3300
	Fine- grained	320 (170[b])	260 (230[b])	2500	6600
Industrial	Coarse-grained	330 (240[b])	260	1700	3300
	Fine- grained	320 (170[b])	260 (230[b])	2500	6600

[a] Assumes contamination near residence

[b] Assumes protection of ground water

1.5 Remediation Strategies for the Reduction of Total Petroleum Hydrocarbons in the Environment.

Hydrocarbon contaminated regions are considered today as a serious environmental problem (SHARMA). Remedial standards for petroleum hydrocarbon contaminated surfaces and sub-surfaces have been developed with the purpose to establish guidelines for environmental consultants. The results of feasibility and treatability studies usually done on the polluted land are often used to define the most suitable remedial strategies and technologies to implement. These guidelines also help to regulate and understand the extent of the contamination according to the exposure schemes, site characteristics, land use pattern, physical conditions of the sand and toxicity of the contaminants (CCME, 2008, 2010). PAHs are assessed and managed separately due to their different physical and chemical properties in comparison to the hydrocarbon fractions (ATSDR, 2011c).

The extent of contamination does not only depend on the chemical composition of the contaminant but also on the physicochemical properties of the sand and land use. This determines the most efficient remediation methods that would be required for an efficient clean-up. Therefore, knowledge of the general properties such as moisture content, pH, textural class etc. of the surrounding sand is required. Generally, sand is the composed of small pieces of rocks with a gritty texture. Sand is made up of unconsolidated small granular materials consisting of grains of rock or mineral particles defined mainly by size, being finer than gravel and coarser than silt and ranging in size from 0.06 mm to 2 mm**.** It is mainly composed of silicate minerals and silicate rock granular particles usually quartz. Sandy soil on the other hand, is composed of about 35% sand and less than 15% silt and clay (Al jaloud, 2013, Ehlen, 1993).

The two major types of remediation strategies are containment and treatment methods. Containment methods aim to limit the movement of spilled oil as well as reducing the hazardous effects. These methods include isolation of spilled oil, control, and stabilization of oil. However, the treatment methods aim to remove the hydrocarbon contaminants from the spill area with some approaches aiming at destruction as well (Dave, Ghaly, 2011).

1.5.1 Treatment Strategies.

The treatment of contaminated soil can be described as a chemical, physical, biological, thermal or a combination process (Masakorala, Yao *et al.*, 2014), (Silva, Rita de Cássia FS, Almeida *et al.*, 2014). The aim is to reduce the total amount of oil as well as to reduce the mobility of the oil in the environment to reach an accepted level (Dave, 2011). Different types of soil treatments can remove different types of contaminants. Some techniques destroy contaminates, while other techniques separate contaminants from the soil without changing them chemically. Therefore, some techniques have a higher potential in removing contaminants from soil, while some are time consuming and not cost effective in treating the desired level of contaminants.

1.5.1.1 Natural Strategies.

Natural methods are based on the use of natural processes such as evaporation, sedimentation, oxidation and biodegradation in order to reduce the spilled oil by removing and degrading the contaminants without further actions. Naturally, the chemical composition of oil can be changed by UV light radiated from the sun and chemical reactions with oxygen in the air (Reed, Johansen *et al.*, 1999).

Bioremediation is the use of different types of microorganisms to degrade and metabolize contaminants. Biological agents such as fungi, bacteria, and phytoremediation (green plants), in order to either neutralize or remove the contaminants from sand or water. Microorganisms (bacteria and fungi) found in sand usually use petroleum hydrocarbons as a source of food, breaking them down in into less hazardous compounds. They also aid in the degradation of compounds such as alcohols, esters, carbonyl compounds, phenols, or naphthenic acids into water and carbon dioxide (Maletić, Dalmacija *et al.*, 2013).

The spilled oil quantity, type of oil, characteristic, availability of oxygen and macro-nutrients (phosphorus, and nitrogen) are important factors that can affect the efficiency of biodegradation. Environmental conditions such as pH, temperature, and salinity also play a major role (Xueqing Z hu, *et al.*, 2001; Maletić, Dalmacija *et al.*, 2013). The spilled oil concentration can affect the biodegradation level for example, if it’s low it might be insufficient to catalyse biodegradation, but if it’s too high it could inhibit the biodegradation processes, due to the unavailability of nutrients (P and K) and oxygen required by the organisms (Maletić, Dalmacija *et al.*, 2013). Availability to microorganisms for breakdown is limited though due to the low water solubility of the contaminants (Silva, Rita de Cássia FS, Almeida *et al.*, 2014).

Alkane compounds C_{10} to C_{20} (intermediate length) are preferred substrates for degradation, while short, straight chain n-alkane compounds are more toxic. Long chain (C_{20}-C_{40}) n-alkanes (waxes) are hydrophobic solids and not easily degraded because of their poor availability and water solubility and branched alkanes degrade slower than straight chain n-alkanes. The degradation rate of cycloalkane is rather variable by several microbial species, but degradation rate is still slower than n-alkanes.

Polycyclic aromatic hydrocarbons (PAHs) do not degrade easily under natural conditions. They are highly resistant toward biodegradation and have recently gathered significant importance due to their carcinogenic and ability to bio-accumulate (Haritash and Kaushik, 2009). However, some microorganisms can degrade aromatic compounds. A major factor which affects the degradation of PAHs is molecular weight. It is reported that PAH with the highest boiling points, those with condensed aromatic and cyclo-paraffinic structures, and bitumen (asphaltic materials) (Haigh, 1996) have the greatest resistance to biodegradation.

Plants can also degrade petroleum hydrocarbons by taking up PAHs in different ways (**Figure 4**). Firstly, PAHs can be taken up through root tissues from the soil. Secondly, PAHs can be adsorbed onto the root surface. Thirdly, PAHs volatilised from the soil surface can be taken up by the leaves and fourthly, PAHs from the atmosphere can absorbed through the leaves' surface (Collins *et al.*, 2006).

A total petroleum hydrocarbon (TPHs) content of 10,000 to 50,000 ppm is however considered inhibitory and toxic to most microorganisms and plants (US EPA, 2009b; Megharaj *et al.*, 2011). For this reason, alternative strategies have been developed.

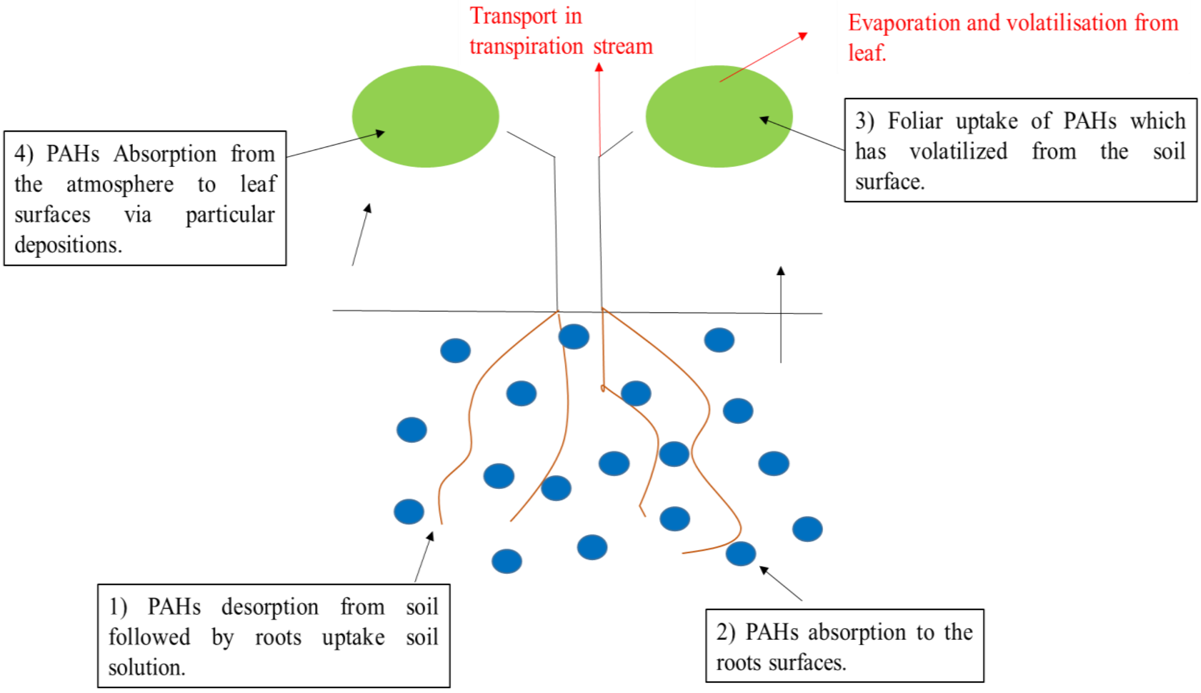


Figure 4 Principal pathways for plant uptake of PAHs (Adapted from Collins *et al*, 2006).

1.5.1.2 Alternative Strategies.

Physical methods are commonly used when large spills occur. It is the first response that is used in the clean-up of most oil spills. In aqueous environments methods such as booms, adsorbents, barriers, and skimmers are used. However, this method can produce contaminated waste during the recovery process (Mohammed 2004, Hamby 1996).

Thermal methods involve oil burning with specialized equipment such as fire resistant booms, and igniters. Burning oil can be performed in calm wind conditions, and with light oil products, which can be burnt easily without damaging the environment (Dave, Ghaly, 2011). In order to destroy oil contaminants in soil (in the case of Saudi Arabia sandy soil), a high temperature range from 400 ^oC to 1000 ^oC is used to volatise and burn contaminants of crude oil in soil. This method can remove petroleum hydrocarbons with high efficiency and is considered as an effective technique because it reduces the crude oil concertation in soil. However, it is an expensive and can lead to the production of hazardous waste.

Chemical methods are an effective method for oil spill remediation. It involves the use of chemical materials such as dispersants, solidifiers, and surfactants. Chemical methods break up and stabilize spilled oil, thus enhancing the rate of the oil biodegradation processes. Solidification and stabilization reduce the mobility of contaminants, crude oil and harmful substances in sand. The composition of oil, oil type, slick thickness, quantity of dispersants, water temperature, and salinity are factors that can influence the effectiveness of chemical methods. The dispersant oil ratio (DOR), which is the amount of dispersant used in the spillage area is highly dependent on the quantity and quality of spilled oil (Dave, Ghaly 2011; Michel, Shigenaka *et al.,* 1992).

The efficacy of both chemical and physical process in the total remediation process is approximately 52%, biological processes (28%), thermal treatment processes (18%) and off gas technology (2%) (Urum, 2004) (USEPA, 2000). In general, physicochemical methods can remove a considerable amount of spilled oils speedily; although spilled oil can transfer contaminants from one environment medium to a different one and produce harmful by-products. Thus, crude oil removal is a challenge using physicochemical methods only (Silva, Rita de Cássia FS, Almeida *et al.,* 2014). Therefore, to achieve optimum levels of remediation, these treatment methods need to be combined.

1.5.2 Soil Washing.

Soil washing uses a combination of physical and chemical strategies to separate and remove contaminants (petroleum hydrocarbons), thereby reducing the concentration of the pollutant in the soil. Soil washing is the desorption of contaminants from soil particles by using mixtures of several organic solvents (i.e. ethanol, methanol, dichloromethane) or surface-active agents such as surfactants. Studies have shown that the soil washing technique is a cost effective and less time-consuming technique compared to bioremediation and phytoremediation, with a capacity to treat and recover a large proportion of contaminants from soil. The clean soil can then be used for any purpose, while the extracted oil can be recycled. Soil washing is most effective for soils that have at least 50% gravel/sand content (i.e. sandy soil).

The United States of America have used soil washing techniques in order to remediate some petroleum hydrocarbon contaminated sites. Studies have shown that soil washing using surfactant solutions have been most effective in removal of PAHs and total petroleum hydrocarbons from contaminated soil including sandy soil, which is the majority of the soil found in Saudi Arabia.

1.6 Surfactants for Remediation.

Surfactants are the active components of detergents, soaps, pharmaceuticals, food and packaging. Surfactants are chemicals that can influence the properties of fluid surfaces because they contain both hydrophobic and hydrophilic moieties and are thus amphipathic (**Figure 5**). The hydrophilic moiety is defined as the head of surfactant and it is water-loving The hydrophobic moiety consist of a long hydrocarbon chain (Rosen, 2004) and repels water (non-polar).

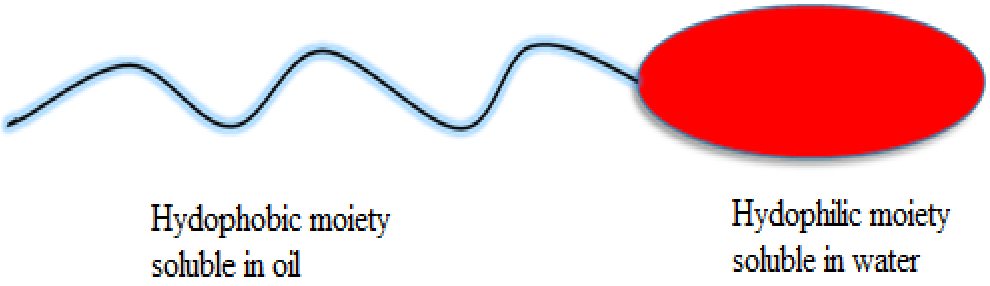


Figure 5 Surfactants molecule with hydrophilic (polar) and hydrophobic (apolar) moieties (adapted from Santos, Rufino *et al.*, 2016).

The hydrophilic moiety can be either carbohydrate, alcohol, cyclic peptide, amino acid, or phosphate carboxyl acid. The hydrophobic moiety can be an ester or protein and it has a long chain of fatty acid.

Surfactants are generally classified into four main classes (anionic, cationic, amphoteric, and non-ionic) based on the nature of the hydrophilic moiety (head of surfactants) (**Figure 6**), but they can also be classified based on their origin, for example:

- Synthetic surfactants which are manufactured and are mainly petroleum-derived. Some common examples include; Triton X, Brij 35, Tergitol NPX and
- Biosurfactants, which are produced from biological-based materials or simply microbes. They are amphiphilic and are synthesised from a diverse range of compounds mainly produced by hydrocarbon-utilizing microorganisms such as bacteria or yeast, but are also available from plants, animals and human sources. They

are biodegradable, and show less toxicity compared to chemical surfactants. Some common biosurfactants include; Rhamnolipid, Lecithin etc, (Paria, 2008).

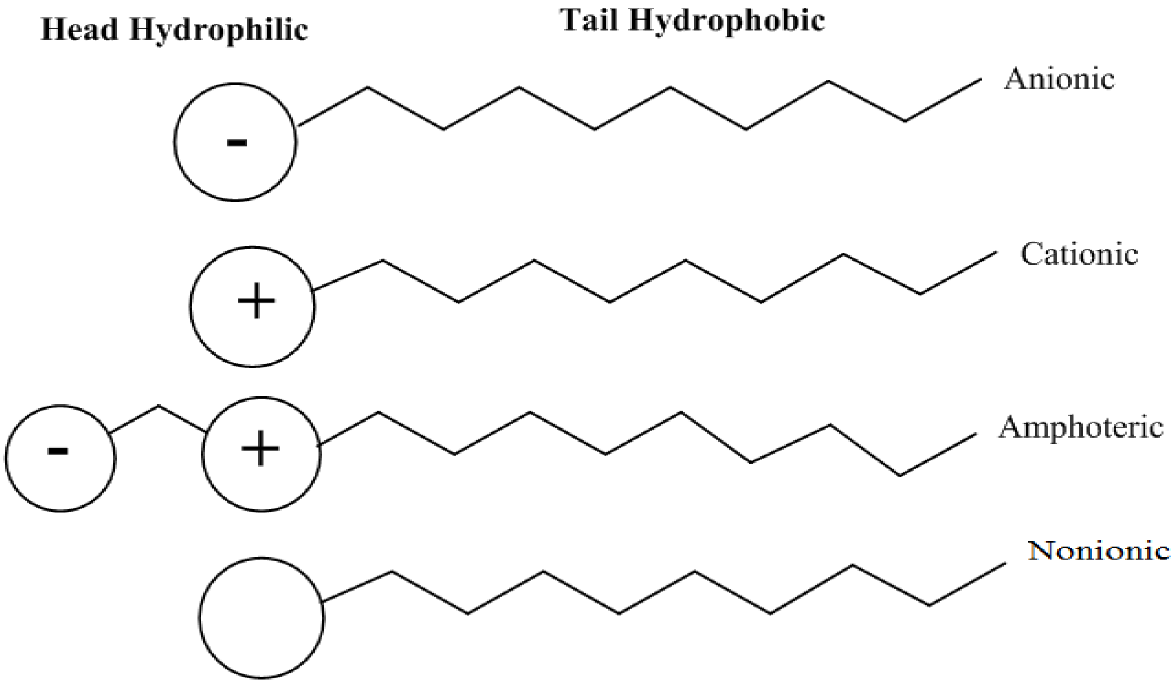


Figure 6 Four classes of surfactants.

Anionic surfactants are one of the most commonly used surfactants and consist of a negatively charged (-) polar head group and a positively charged counter ion. These include fatty acid soaps ($RCOO^-Na^+$), sodium dodecyl sulfate (SDS) (**Figure 7**), alkyl-benzene sulfonate ($RC_6H_4SO_3^-Na^+$) and lauryl sulfate. This type of surfactant is most effective on positively charged surfaces (Rosen, Kunjappu, 2012).

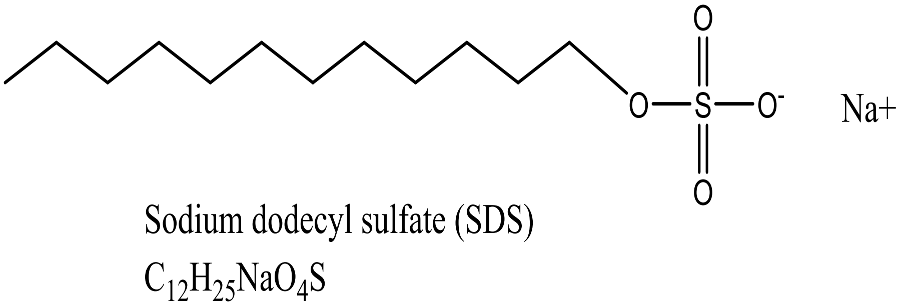


Figure 7 Chemical structure for sodium dodecyl sulfate.

Cationic surfactants consist of a positively charged (+) polar head group and have a negatively charged counter ion. They include salts of long chain of amines ($RNH_3^+Cl^-$), and quaternary ammonium chloride ($RN(CH_3)_3^+Cl^-$) (Rosen 2004).

Amphoteric surfactants contain both negative (-) and positively (+) charged groups on the polar head. They include long chain amino acids ($RN^+H_2CH_2COO^-$), and sulfobetaine ($RN^+(CH_3)_2CH_2CH_2SO_3^-$) (Rosen, 2004). Amphoteric surfactants can be adsorbed onto both negative and positively charged surfaces because they bear both charges (Rosen, Kunjappu, 2012).

The non-ionic surfactants have no ionic charges on the polar head and include monoglyceride ($RCOOCH_2CHOHCHOH$) and polyoxyethylenated alcohol ($R(OC_2H_4)xOH$). The non-ionic surfactants can be adsorbed to either hydrophilic or hydrophobic moieties depending on the nature of the surface. For example, if a polar group undergoes hydrogen bonding with the polar moiety of the surfactant, the surfactant is adsorbed with the hydrophilic group oriented to the surface, exposing the hydrophobic moiety. However, surfactants can also be oriented with their hydrophobic group facing the surface and exposing the polar group (Rosen, Kunjappu 2012; Rosen, 2004) .

1.6.1 Surfactant Properties.

Surfactants are designed to carry out specific functions. Not all surfactants are equal and interchangeable, and the specific application determines the choice of surfactant. Surfactant properties such as the Hydrophilic lipophilic balance (HLB), critical micelle concentration (CMC), biodegradability, toxicity, surface and interfacial tension determine the suitability of surfactants for soil washing of polluted areas.

1.6.1.1 Critical Micelle Concentration (CMC).

At low concentrations, surfactants are found as either monomers or single molecules (Bustamante, Durán *et al.*, 2012). The critical micelle concentration (CMC) is defined as the minimum concentration of surfactant in solution at which surfactant monomers form a spheroid structure. As the monomers begin to accumulate, aggregates are formed known as micelles. This occurs because of changes in the physicochemical properties of the solution such as reduction in the interfacial tension between surfaces, enhanced mobility and conductivity (**Figure 8**).

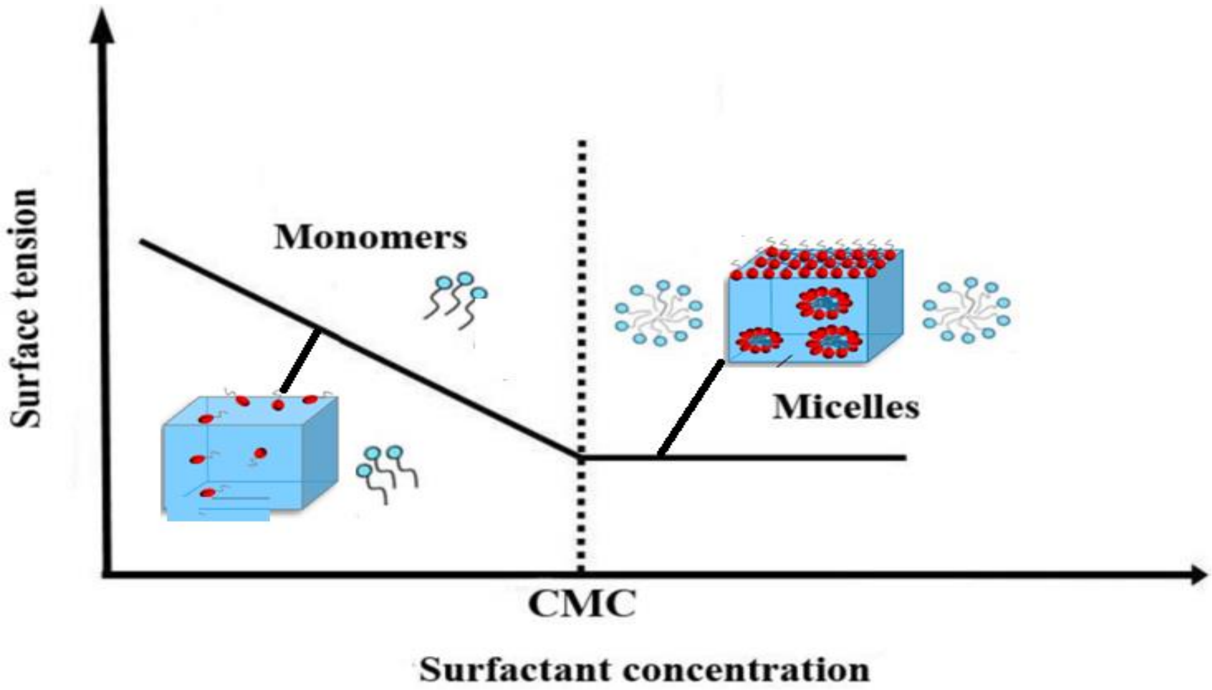


Figure 8 Clarification of tension-active agent and micelles formation (Adapted from Santos, Rufino et al. 2016)**.**

However, when the concentration of the surfactant is too high, there is no more space for the surfactant molecules on the water surface, impeding micelle formation. Some physical properties which affect the CMC of a surfactant include the ionic characteristic, chemical structure and temperature (Maletić, Dalmacija *et al.*, 2013). CMC is also highly dependent on salinity, surfactant type and hydrocarbon chain length (Bustamante, Durán *et al.*, 2012). The type of the contaminant in solution also significantly affects the CMC. Hydrophobic contaminants can be dissolved in their hydrophobic core by the micelles, which might cause an increase in aqueous solubility of the pollutant (Santos, Rufino *et al*., 2016);(Bustamante, Durán *et al.*, 2012).

Surfactant solubilisation is a crucial characteristic, which is related to micelle formation. Solubilisation is the dissolution of a substances (solid, liquid, or gas) in a solvent when micelles interact with substances in order to form a thermodynamically stable solution. The rate of surfactant solubilisation is an important factor in the evaluation of a surfactant's ability to remove hydrocarbons from contaminated sand or water. The solubility of solvent insoluble material, which is plotted against surfactant concentration, will be low until it reaches the CMC. After reaching the CMC, the solubility of an otherwise insoluble material will increase with increasing surfactant concentration. Thus, hydrophobic organic compounds can be solubilised by surfactants above the CMC of that surfactant (Rosen, 2004).

Previous studies have shown that the CMC of surfactants can range from 1 to 2000 mg/L (Sobrinho, Luna *et al*., 2013, Silva, Rita de Cássia FS, Almeida *et al.*, 2014). Lower CMC indicates greater efficacy of the surfactants, which can favour industrial processes (**Table 4**) (Bognolo, 1999).

Table 4 Some examples of CMC of chemical surfactants (Bognolo, 1999).

Surfactant	Type	CMC (mg/L)
Polyoxyethylene sorbitan monostearate (Tween 60)	Nonionic	30.13
Polyoxyethylene lauryl ether (Brij 35)	Nonionic	1.86
Sodium N-dodecyl sulfate (SDS)	Anionic	230.4
Diphenyl oxide disulfonates (DOSL)	Anionic	321
Alkylbenzene sulfonate	Anionic	590
Sodium lauryl sulfate (SLS)	Anionic	2000 to 2900

1.6.1.2 Interfacial and Surface Tension.

Surface tension is the tension between air and water, whereas interfacial tension is the tension between oil and water. Both surface and interfacial tension can be reduced by surfactants which can leads to solubilisation, emulsification, phase dispersions, and lubrication. Surface tension is considered as the most crucial characteristic of bio-surfactants (tension-active agent).

The capillary forces which hold the non-aqueous phase liquid (NAPLs) in sand can be reduced by reducing the interfacial tension of oil using surfactants. Therefore, this characteristic can increase the mobility of oil in sand as well as enhance the separation from porous media. It has been reported, that the selection of surfactant solutions for sand washing is an important point to consider. A surfactant, which provides effective solubility of oil and adequately reduces the interfacial tension, is preferred (Brown, Pope *et al.*, 1994).

1.6.1.3 Hydrophilic Lipophilic Balance (HLB).

The hydrophilic-lipophilic balance (HLB) is a measure of the solubility and behaviour of surfactants solutions in water. The balance HLB number is a significant factor and ranges from 0 to 20 depending on the correlation of hydrophilic and hydrophobic parts of the surfactants. An HLB of 0 indicates that the surfactant is mostly hydrophobic (lipophilic) molecule and more soluble in oil, while a number of 20 indicates the surfactant is very hydrophilic (water-loving) (**Figure 9**). The surfactant with a high HLB can be used in order to form oil in water emulsions, while the surfactants with low HLB are insoluble in water and form water in oil emulsions.

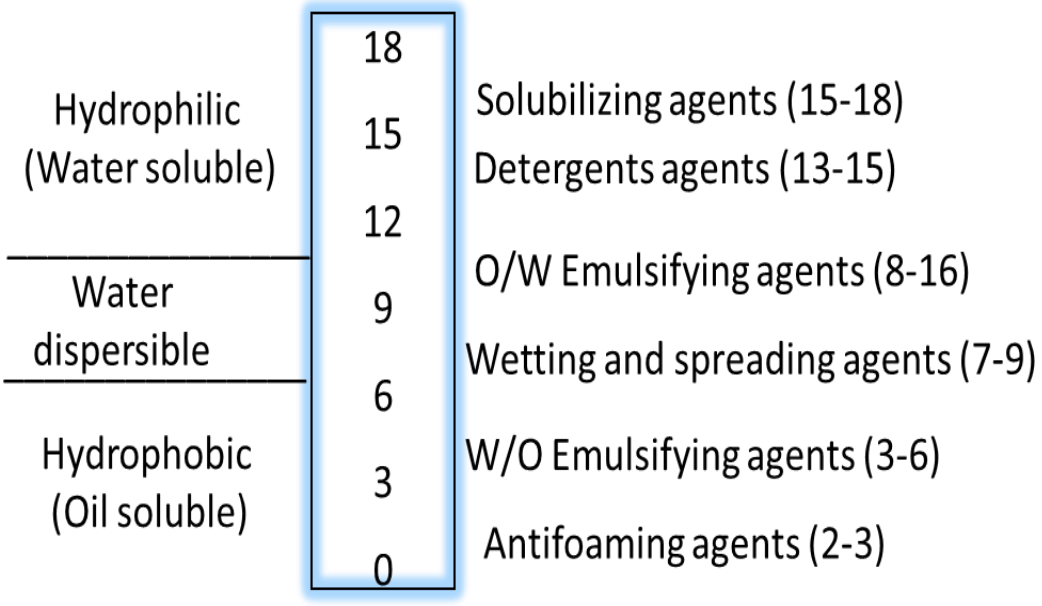


Figure 9 HLB range illustrates surfactant functions classification.

1.7 Objective of the Present Project.

Saudi Arabian sandy sands are prone to contamination by petroleum hydrocarbons because of the petrochemical activities such as drilling, pumping, transport, storage and refining and historic conflicts. Therefore, evaluating a potential remediation approach for petroleum hydrocarbon contaminated sites is of great importance. This research addresses the potential for the remediation of petroleum hydrocarbons from sands taken from different locations in Saudi Arabia (Riyadh, Dammam, Khafji, Sulayyil, Karj, Hufuf, Qatif, Unaizah, Buraidah, Skaka, Tabuk, Rafha, Jeddah, Najran, Alhassy, and Khobar).

Firstly, 16 different sands from Saudi Arabia will be analysed for total hydrocarbon content. Some of the samples such as Kafji (KF), Dammam (D), Qatif (Q), Hufuf (H) and Sulayyil are expected to already contain hydrocarbons, because they located near oil refineries. In addition, no publicly available survey of background concentrations of hydrocarbon in Saudi desert environments has been found. This project aims to separate, identify and quantify the aliphatic and aromatic hydrocarbon fractions using glass column chromatography. Next, the ecotoxicology of hydrocarbon pollution will be determined by measuring the effects of increasing hydrocarbon concentrations on seed germination. Last, but not least, surfactant washing will be investigated as a remedial strategy for polluted sand. At least three “off-the-shelf” consumer surfactants will be tested for their ability to remove hydrocarbons from diesel and crude oil spiked samples and the total amount of hydrocarbons remediated will be quantified.

In this project, the GC-MS technique is used to achieve the following aims:

- To quantify and identify the aliphatic and aromatic moieties present in Saudi sand samples and.
- To quantify the total petroleum hydrocarbons remediated from spiked Saudi sands.

2 Chapter 2: Background Survey of TPHs Concentrations in Saudi Arabian Sands.

2.1 Introduction.

Sandy soils are referred to in general terms as coarse textural soils. They are usually grouped into basic structural textual classes such as sands, loamy sands, sandy loams, fine sands, coarse sands, loamy coarse sand, dune sands, gravelly sand, gravelly loamy sand and various subdivisions occurring predominately in the arid and hyper-arid regions subtropical climatic zone of the Arabian Peninsula. This classification is based on the particle size distribution as follows: gravel (>2 mm), very coarse sand (1-2 mm), coarse sand (0.5-1 m), medium sand (0.25-0.5 mm), fine sand (0.125-0.25 mm), very fine sand (0.075-0.125 mm), and silt clay (<0.075 mm) (Ehlen, 1993). A recent study to evaluate the environmental risks of sand dunes in Khulays dune field showed that the particle size distribution of the tested dune sands showed that samples were predominantly classified as medium sand (17.5 - 24.5%), fine sand (33.4 - 38.4%) and very fine sand (16.8 - 45.1%) with a very small percent of coarse sands (0.9 - 2.2%) and fines (silt and clay-sizes particles) around 2% (Sonbul, 2016). Al jaloud, 2013 reported the textual class of seven samples taken from agricultural regions of Saudi Arabia; Kharj, Delim, Qassim, Hail, Jouf, Wadi D and Taif as sand, clay loam, sandy clay loam, sandy loam, sandy loam, sand and loam respectively.

The presence of large number of pores is responsible for the good aeration, fast drainage and low moisture holding capacity. The low water holding capacity of sandy soils usually affects its moisture content, as sandy soils are low in clay content most of the pore are relatively large with the available water. Berlin et al. 1986 reported that the soil moisture in the Al Labbah sand in northern Saudi Arabia to be between 0.15 - 0.25 % at 20-30 cm depth and 0.18 - 0.58 % at 50 cm depth. Surface soil moisture was also reported to as range from 0.054 % to 0.077 % (Berlin et al. 1986). In 2015 Khan, Hussein *et al,* reported that the soil moisture in some Saudi sands ranged from 0.52 % to 1.2 %.

Other chemical properties of sandy soil such as pH has been reported to be generally slightly alkaline due to its calcareous nature. Al Jaloud, 2013 reported the pH of seven samples taken from agricultural regions in Saudi Arabia; Kharj, Delim, Qassim, Hail, Jouf, Wadi D and Tif and ranged from 7. 7 - 8.1 across all the geographic regions. Yasir, Azhar *et al.* (2015) reported that pH ranged from 7.7 to 8.3 for ten Saudi sand allocated in southwestern highlands of Saudi Arabia.

Other chemical properties of sandy soil such as organic matter has been reported to be generally low ranged from 0.21 % to 1.03 % (Hashem 1993). Yasir, *et al.* (2015) reported that the organic matter content of Saudi sand samples ranged from 0.21 % to 1.75 %. In addition, the organic matter for Saudi sand in Riyadh city was reported to be 0.43 % by Al-Oud, Nadeem *et al.* (2011).

Sandy soils are generally poor in plant nutrients, this is mainly attributed to their low fertility as well as loss of water and nutrients as a result of leaching. The invariability of the aeolian sediments from wind-blown sand pile up to form sand dunes which seriously impair the crop growing areas and reduce the efficient of the irrigation and drainage networks. The agricultural potential of sandy soils in arid regions like Saudi Arabia is also largely hampered by their low water holding capacity which results in leaching of applied nutrients in particular nitrogen, weak structures and as stated earlier low organic matter content.

The Arabian Desert consists of two major regions. The first, the ancient Arabian platform (a segment of the African Shield), is in the west. The second region, in the east, comprises sedimentary rock layers deposited on continental shelves and within marine basins along the margins of the Arabian platform. Vast amounts of petroleum formed between those sedimentary rock layers, making the Arabian Desert the richest petroleum-producing region in the world. Apart from having a natural reserve of crude, petroleum contamination of soil caused by transportation, accidents and leakages have brought serious environmental damage to the region. Therefore, an accurate and reliable technique, which can be used for the analysis of crude oil, and oil-spill-related environmental samples, is extremely important. The most commonly used method for crude oil separation, characterization, and identification involves the use of a capillary GC-MS . Crude oil and oil-spill-related samples are extremely complex mixtures in which the boiling points of components can vary over a wide range therefore, the complete separation of such complex samples into individual components is difficult or impossible even with high-resolution capillary GC. Therefore, a good, efficient and cost efficient method of fractionation is required to separate the compounds into classes based on similarity in structure. The most widely used technique in the separation of crude oil derived materials include liquid-liquid extraction and high-performance liquid chromatographic separation. For reasons of cost, ease, and efficiency of operation, separation of petroleum hydrocarbons is commonly performed using the conventional adsorption chromatography on various adsorbents, including silica gel, alumina and silica-alumina combinations (Wang et al., 1994).

The use of solid phase extraction (SPE) as an alternative technique to fractionate petroleum and to isolate polycyclic aromatic hydrocarbons (PAHs) from samples has been reported (Wang et al., 1994). However, using commercial SPE cartridges for the fractionation of petroleum have several disadvantages such as: fixed adsorbent quantities, no variability of activity, and the possibility of the introduction of interferences from polyethylene cartridge housings and frits. Wang et al., 1994, reported the development and use of a simple and reliable silica gel liquid chromatographic technique using inexpensive, home-packed silica gel micro glass columns for the fractionation of a synthetic n-alkane and PAH standard mixture into aliphatic and aromatic hydrocarbons. The silica gel liquid chromatographic method, combined with capillary GC, was also reported to be successful for the identification, characterization, and quantitation of hydrocarbon components in oils and complex oil-spill-related environment samples.

Saudi Arabia is one of the richest countries in the world in terms of unconsolidated sand and sandstone reserves (Garzanti et al. 2003). The majority of the soil in the kingdom of Saudi Arabia are sandy soils, due to the arid nature of the climate. This chapter aims to investigate the physiochemical properties and characteristics (elemental composition using ashing, aqua regia and nitric acid digestion) of 16 Saudi sand samples from different geographical region of Saudi Arabia. Samples were collected from Riyadh, Dammam, Khafji, Sulayyil, Karj, Hufuf, Qatif, Unaizah, Buraidah, Skaka, Tabuk, Rafha, Jeddah, Najran, Alhassy, and Khobar. Saudi Arabia also has large reserves of crude oil and because of this; it is highly prone to petroleum contamination of soil and water caused by transportation, accidents and spillages. In addition, hydrocarbons can also be present naturally as background. In this chapter, the water and DCM extractable fractions of petroleum hydrocarbon in the 16 Saudi sand samples were determined. A method to separate, identify and quantify the aliphatic and aromatic hydrocarbon fractions using a glass column chromatographic separation method was also established.

2.2 Materials and Methods.

2.2.1 Materials and Methods.

All chemicals were of reagent grade and were used without further purification. Analytical grade hexane and dichloromethane (DCM) were purchased from Fisher Scientific UK. Sand samples were collected from sixteen different cities in the Kingdom of Saudi Arabia. All of the working solutions were prepared at concentrations ranging from 50 ppm to 950 ppm from 1000 ppm stock solution in hexane. Alkane standard mixture of 13 aliphatic hydrocarbons from C_8 to C_{20} and certified alkane standard mixture of 34 aliphatic hydrocarbons from C_7 to C_{40} were purchased from Sigma (numbers in parenthesis are equivalent to carbon number) at a concentration of 40 ppm (mg/L) and 1000 ppm (mg/L) in hexane (**Table 5**). Diesel oil (commercial petroleum) was purchased from petrol station in North Wales (Esso Tesco Chelsea Express). Crude Oil was donated from Dr Charlie Shand, James Hutton Institute, UK. Anhydrous sodium sulfate and silica gel were obtained from Fisher Scientific UK. Aromatic standard 2000 ppm, which contains 16 compounds, was purchased from Fisher Scientific UK. 5 α-Androstane (internal standard) was purchased from Fisher Scientific UK.

Table 5 Certified alkane standard mixture of 34 aliphatic hydrocarbons from C_7 to C_{40}.

Carbon number	Hydrocarbon Name	Carbon number	Hydrocarbon Name
C_7	n-heptane	C_{24}	n-tetracosane
C_8	n-octane	C_{25}	n-pentacosane
C_9	n-nonane	C_{26}	n- hexacosane
C_{10}	n-decane	C_{27}	n-heptacosane
C_{11}	n-undecane	C_{28}	n-octacosane
C_{12}	n-dodecane	C_{29}	n-nonacosane
C_{13}	n-tridecane	C_{30}	n-triacontane
C_{14}	n-tetradecane	C_{31}	n-hentriacontane
C_{15}	n-pentadecane	C_{32}	n-dotriacontane
C_{16}	n-hexadecane	C_{33}	n-tritriacontane
C_{17}	n-heptadecane	C_{34}	n-tetratriacontane
C_{18}	n-octadecane	C_{35}	n-pentatriacontane
$9C_{19}$	n-nonadecane	C_{36}	n- hexatriacontane
C_{20}	eicosane	C_{37}	n-heptatriacontane
C_{21}	n-heneicosane	C_{38}	n-octatriacontane
C_{22}	n-docasane	C_{39}	n-natriacontane
C_{23}	n-trcosane	C_{40}	n-tetratriacontane

2.2.2 Sample Collection.

Sixteen Saudi sand samples (4.5 kg per city) were collected from different location from Kingdom of Saudi Arabia in order to find out if there was a difference in the quantity of hydrocarbon in sands. The sixteen location are illustrated in the following map **(Figure 10)**.

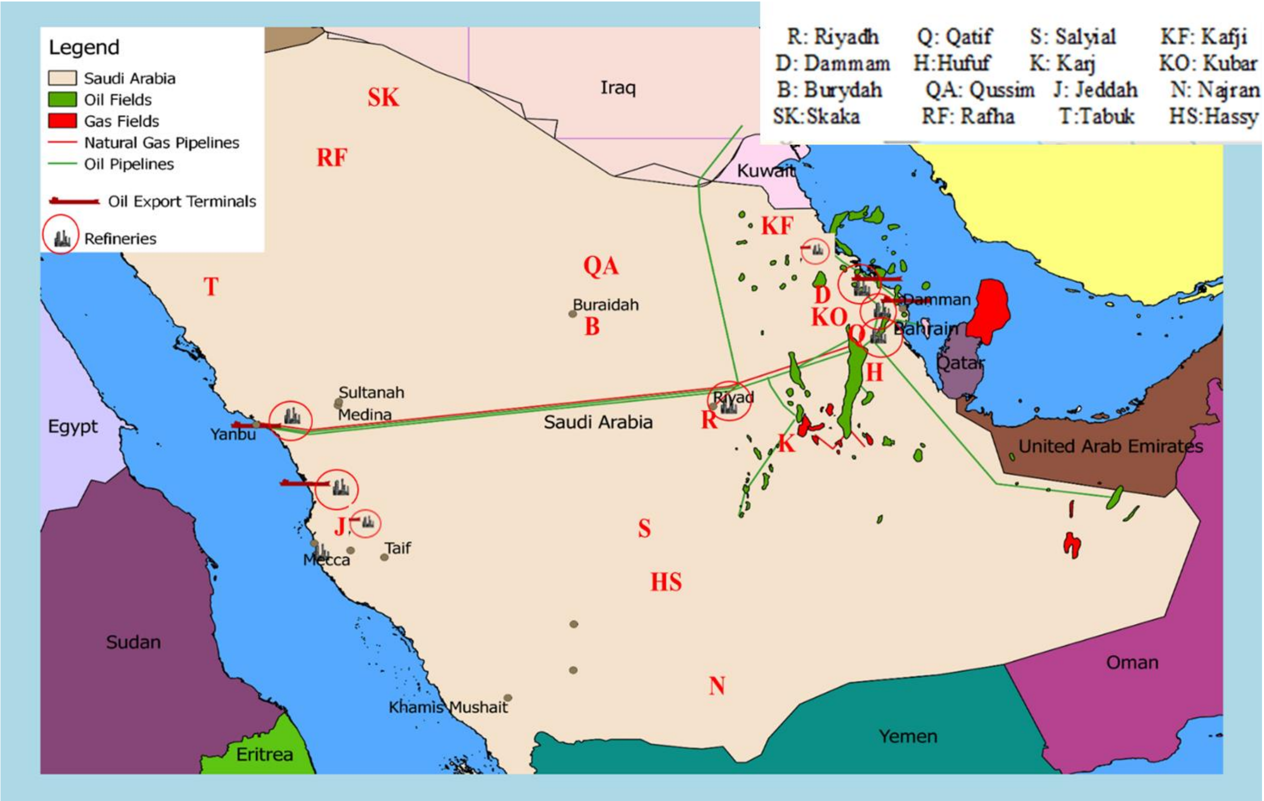


Figure 10 Image of Kingdom of Saudi Arabia showing locations of collected sand samples.

Samples were shipped from Saudi Arabia (Riyadh) to the UK via DHL in plastic container. In order to present the samples easily in graphs and bars, they are referenced as K, KF, D, H, Q, QA, B, R, KA, SK, RF, T, J, N, HS and S (**Table 6**). Samples were collected at various depth ranged approximately from the surface to 30 cm.

Table 6 Saudi Arabian sands location and cities names.

Abbreviated Saudi Name Cities	Saudi Sample Cities Name	Collection Date	Location
D	Dammam	01.09.2016	East of Capital city
K	Karj	01.09.2016	South of Capital city
H	Hufuf	01.09.2016	East of Capital city
S	Sulayyil	01.09.2016	South of Capital city
KF	Khafji	01.09.2016	East of Capital city
R	Riyadh	09.10.2016	The Capital city
Q	Qatif	09.10.2016	East of Capital city
KO	Khobar	09.10.2016	East of Capital city
AN	Unaizah	10.11.2016	West of Capital City
B	Buraidah	10.11.2016	West of Capital City
SK	Skaka	06.02.2017	North of Capital City
T	Tabuk	06.02.2017	North of Capital City
RF	Rafha	07.02.2017	North of Capital City
J	Jeddah	01.02.2017	West of Capital City
N	Najran	11.02.2017	South of Capital City
HS	Al-Hassy	09.02.2017	South of Capital City

2.2.3 Type of Sands.

Sixteen different sands (72 kg in total) were collected from Saudi Arabia. The sand was smooth and had small grains. The colour of sand differed across the sample however, most of them were near to red in colour and some were white and brown-red in colour. The sand was used without further processing or cleaning.

2.2.4 Sample Storage.

All sand was stored at room temperature and kept in closed air-tight containers in order to avoid contamination and water absorption.

2.2.5 Control Sand.

A horticultural sand was purchased from Treborth Garden Centre, which was used as a control sand (CS) in all experiments in all experiments reported in this thesis. Total petroleum hydrocarbon determination by soxhlet extraction, DCM and deionised water shaking for 24 h showed that there was no hydrocarbons in horticultural sand sample. The pH, percentage of carbon, nitrogen, organic matter and moisture content of the CS was determined to be 0.01%, 0.02%, 7.9, 0.3% and 0.1%, respectively. Elemental analysis of the CS showed that it contained 600 mg/kg (Al), 912 mg/kg (ca), 205 mg/kg (K), 249 mg/kg (Mg), 800 mg/kg (Na) and 22 mg/kg (P).

2.2.6 Instrumental Analysis.

Apparatus:

Chromatographic analysis was performed using a 6890N Agilent Technology Network GC System with mass spectrometer equipped with Voyager mass spectrometer Thermoquest-Finnigan MS. The instrument setting and parameters were stated in **table 7**.

Table 7 Instrument setting and parameters for GC-MS.

Column Type	DB-5	**Column Dimension**	30 m, 0.28 mm ID and 0.25 μm film thickness
Temperature Limits	-60 °C to 325 °C	**Transfer Line Temperature**	290 °C
Injection port Temperature	270 °C	**Detector Voltage**	350 V
Column inlet pressure	15 p s I g	**Mass Spectrometry Detector**	Operated in full scan mode from 50 to 550 D
Carrier Gas	Helium	**Electron impact EI**	Run in positive mode

For the aliphatic analysis, 0.2 µl of the samples were injected in split-less mode at 250 °C. The temperature program, which was used to analyse samples and standards, are shown in **table 8**.

Table 8 Temperature programme setting used for GC analysis.

Initial Temperature	35 °C for 2 min
Ramp 1	10 °C /min to 250 °C
Ramp 2	20 °C /min 320 °C held for 13 min
Total Run Time	40 min

2.2.7 Standards.

Different standards were prepared for GC-MS and inductively coupled plasma atomic emission spectroscopy (ICP-AES) as described below.

2.2.7.1 Standards for GC-MS.

A 1000 ppm certified alkane standard mixture of 34 aliphatic hydrocarbons from C_7 to C_{40} and aromatic standard of 16 compounds was ran by Gas chromatography–mass spectrometry (GC-MS) using the instrumental setting and method as describes in **table 7 and 8**. A calibration range of 50 ppm to 950 ppm of aliphatic and aromatic standards was prepared and used for quantitative analysis, while a 5 α-Androstanol (50 ppm) was used as an internal standard when analysing samples.

2.2.7.2 Standards for ICP-AES.

For ICP-AES, elemental standards were prepared in order to quantify the amount of certain elements (Al, Ca, K, Mg, Na, P, S, Cu, Fe, Mn, Zn, Sr, Ba,Si and Ni) in sand samples. A set of different elements concentrations were prepared in order to obtain a liner curve. The concentration of standards ranged from 5 ppm up to 140 ppm and were prepared in 10 ml of deionised water.

2.2.8 pH Determination.

10 g of sand samples were placed into centrifuge tubes with 25 ml of deionised water. Then shaken using the plate shaker for one hour at 250 rpm. Samples were left to settle for 30 minutes before shaking again for 5 minutes. The pH was measured for all sand samples by taking the measurement five times, then calculating the average.

2.2.9 Sand Moisture Determination.

Empty crucibles were weighed then 2 g of sand samples added into the crucible. The samples were put into the oven at 105 ^{o}C overnight in order to lose water content. The crucibles were left in a desiccator for 10 minutes in order to cool down before being reweighed. The difference between the sand crucible and empty crucible was the water content for sand sample.

2.2.10 Organic Matter Determination.

Empty crucibles were weighed and then 2 g of sand samples added into the crucible. The samples were heated in a muffle furnace at 550 ^{o}C for 5 hours. The samples were left to cool down for 30 minutes in a desiccator before being reweighed to calculate the organic matter as shown below.

$$\text{Perecentage of Organic Matter} = \frac{\text{Wd} - \text{Wa}}{\text{Wd} - \text{Wt}} * 100$$

Where:

Wd: weight of dry sand sample and crucible.

Wa: weight of ashed sand sample and crucible.

Wt: weight of dry crucible.

2.2.11 Glass Column Chromatographic Separation Method.

A glass chromatography column (20*1 cm) with a frit in the bottom of the column in order to prevent silica from going through the stopcock was rinsed with DCM and then hexane and allowed to dry. The glass column was packed with 3 g of activated silica gel **(2.2.12)** and topped with anhydrous sodium sulfate overlying 0.5 cm. In order to condition the column, 20 ml of hexane was added and the eluent discarded (Wang, Fingas *et al.*, 1994). From 200 µl to

500 µl of crude oil or diesel was quantitatively added onto the glass column prior to exposure of the anhydrous sodium sulfate. 15 ml of hexane was added onto the column in order to elute aliphatic hydrocarbons in a glass vial and the eluent was collected and labelled as fraction 1 (F_1). Then 15 ml of (50% v/v) DCM-Hexane was added into the column in order to elute the aromatic fraction in a glass vial, the eluent was collected and labelled as fraction 2 (F_2). Half of F_1 and half of F_2 were combined together in order to quantify the TPH in a glass vial and the combination was labelled as fraction 3 (F_3). These three fractions were re-concentrated using a nitrogen concentrator to a volume of approximately 0.5 to 1 ml. Then 0.5 ml of the each fraction was transferred into a GC vial in addition to 5 α-Androstane (internal standard) in order to quantify and sent for GC analysis (Wang, Fingas *et al.*, 1994).

2.2.12 Silica Gel Activation.

In order to activate the silica gel before use, 100 g of silica gel was placed into a foil tray and placed in an oven at 130 °C for 16 hours. Before using the silica gel, the oven temperature was increased up to 160 °C for several hours. The silica gel was then used as an activated silica gel.

2.2.13 Determination of percentage of Aliphatic and Aromatics in Crude Oil.

Percentage of aliphatic and aromatic fractions were determined using the same separation method described above (**2.2.11**). Fractions were collected in to pre-weighed glass vials and evaporated until dry under a nitrogen concentrator. The glass vials containing fractions were then re-weighed to calculate the percentage of aliphatic and aromatic fractions from the original crude oil sample.

2.2.14 Determination of Specific Gravity in Crude Oil and Diesel.

First and foremost, an empty glass vial was pre-weighed three times and the average taken. Different volumes of either crude oil or diesel (0.5 ml, 1 ml, 1.5 ml, and 2 ml) was added into each vial. The weight of the liquid was calculated from the difference between empty and filled vials. The temperature was maintained throughout. This was repeated using the same volume (0.5 ml, 1 ml, 1.5 ml, and 2 ml) of water rather than crude oil or diesel. The following formula was used to calculate the specific gravity of crude oil and diesel.

$$Specific\ Gravity = \frac{W_1}{W_{water}}$$

Where: W_l is the mass of crude oil or diesel.

W_{water} is the mass of water.

2.2.15 Leaching Hydrocarbons in sand samples (Water Extractable Aliphatic Compounds).

Water extraction by separating funnels was used in order to measure and quantify the TPHs extractable in sands. Leaching test for sand samples was done by taking three parts water and one part sand (ratio 1:3) in a conical flask and shaking them for 24 h on an electrical shaker. Samples were left for 10 minutes in order to settle, before the supernatant liquid was poured in a beaker. 20 ml of DCM was added into the separating funnel with the water extraction added on top of DCM. The funnel was sealed and shaken for two to three minutes, three times. Between each shaking, the funnel needed venting in order to release excess pressure. The organic extraction was left for three to five minutes in order to separate from the water phase before collecting the solvent extract into a beaker. The extraction was repeated two more times, each time with 20 ml of DCM, follow the same procedure. Then the three fractions were combined together in a beaker. Sufficient amounts of anhydrous sodium sulfate was added into the fractions in order to repel any water. The fractions were filtered into round bottom flasks and evaporated until dry. The residue was then dissolved in 1 ml of hexane and transferred into GC vials, then sent for GC-MS analysis.

2.2.16 DCM Extraction (24 h).

10 g of sand sample was placed into a conical flask and 20 ml of DCM added. The conical flask was sealed by parafilm and left shaking at 250 rpm for 24 h. The fraction was collected in a round bottom flask then evaporated until dry. The residue was dissolved in 1 ml of hexane for GC-MS analysis. (Schwab *et al.*, 1999).

2.2.17 Sample Preparation Methods for Elemental Analysis of Saudi Sands.

The following methods were applied, in order to extract all elements from different sand samples and quantify the amount of the elements. Three different methods were applied for comparison of results and confirmation of the concentrations that were obtained. Elemental analysis were determined by more than one methods, as described in sections (**2.2.17.1 – 2.2.17.3)**.

2.2.17.1 Open Vessel Acid Digestion (HNO_3 70%) (Hot Plate Digestion).

In this method, 1 g of each sample was transferred into clean digestion tubes, to which 5 ml of concentrated nitric acid (70%) was added. The tubes were then allowed to stand overnight in order to prevent the samples from frothing and foaming once heat was applied. The following day, colour changes were noted (brown and red colours appeared). Thereafter, all samples were heated in a digestion block heater at 80 ^{o}C for 30 minutes, after which the temperature was raised to 100 ^{o}C for an hour, then to 120 ^{o}C for the next hour, and finally to 140 ^{o}C for the fourth and final hour. The final step was to leave the samples for approximately 30 minutes in the digestion block heater, in order to cool down. All samples were then diluted to a final volume of 50 ml with deionised water. All samples were filtered through Whatman paper No.42 before the final concentration of elements (Al, Ca, K, Mg, Na, P, S, Cu, Fe, Mn, Zn, Sr, Ba, Si and Ni) in each sample was measured using ICP-AES.

2.2.17.2 Dry Ashing.

A known amount of sample was placed in a crucible and dried in a muffle oven (Carbolite, UK) at 550 °C for 4 h. The sample was allowed to cool down and approximately 0.5 g of ashed sample was dissolved in 5 mL of 70% HNO_3. The solution was diluted with deionised water, filtered and stored at 4 °C before analysis. The final concentrations were determined by multiplying the concentration found in the solution by the ash ratio (mass ashed sample/mass dry sample).

2.2.17.3 Digestion using Aqua Regia.

1 g of sample was added into a digestion tube with 5 ml of HNO_3: HCl (1:3). The tube was sealed and left to stand overnight in order to prevent foaming. Thereafter, all samples were heated in a digestion block heater at 80 ^{o}C for 30 minutes, after which the temperature was raised to 100 ^{o}C for an hour, then to 120 ^{o}C for the next hour, and finally to 140 ^{o}C for the fourth and final hour. The final step was to leave the samples for approximately 30 minutes in the digestion block heater, in order to cool down. All samples were then diluted to a final volume of 50 ml with deionised water. All samples were filtered through Whatman paper No.42 before the final concentration of elements in each sample was measured using ICP-AES.

2.3 Results.

2.3.1 Physio-Chemical Properties for Sands.

The pH across all sand samples ranged from 8.06 to 9.64 with sand samples Buraidah and Skaka having the highest value of 9.64 and 9.37, respectively, while the lowest pH was found in sand Jeddah (8.06) followed by Khafji with 8.1 (**Figure 11**). This shows that all sands are alkaline in nature

Regarding sand moisture, all samples showed similar moisture content, ranging from 0.04% to 0.68% (**Figure 11**). The highest moisture content was found in sand Unaizah (0.68%) followed by Qatif (0.45%) and the lowest moisture content was Rafha (0.04%) followed by Skaka (0.06%).

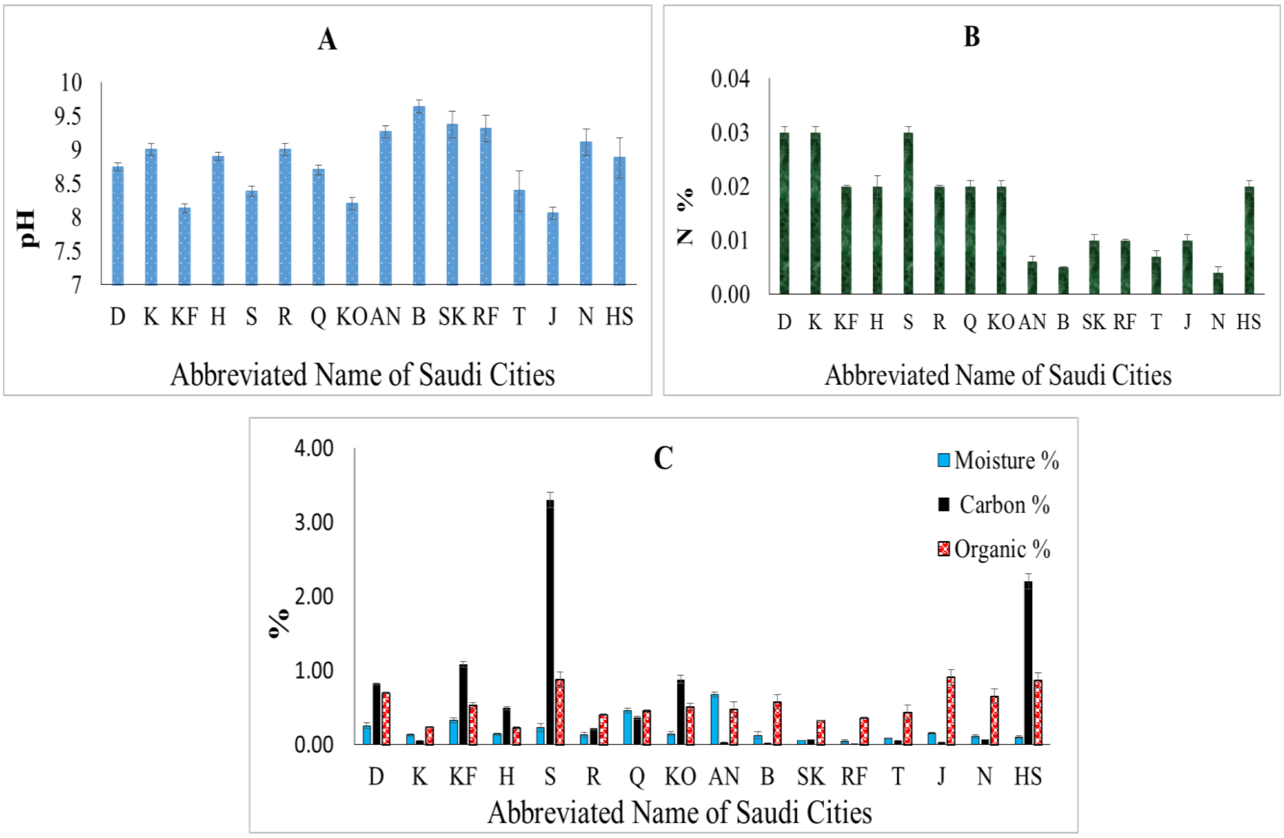


Figure 11 Physio-chemical properties of Saudi sand samples. Graph A shows the pH values. Graph B gives the percentage nitrogen content and Graph C shows the percentage of moisture (blue), carbon (black) and organic matter (red).

Organic matter content in sand samples ranged from 0.22% to 0.91%, this suggests that all samples contain very little organic matter (**Appendix 1**). The highest organic matter content was found in sand Jeddah (0.91%) followed by Sulayyil (0.87%) then followed by HS (0.86%), the lowest organic matter content was found in both Karj and Hufuf, which had a value of 0.22% (**Figure 11**). These results support the relatively low organic matter content of sands from this region as shown by the low organic matter for sands in this study. The sand samples were all collected from desert environments and hence the long absence of plant or animal life is reflected in the low organic matter content.

2.3.2 Elemental Analysis (mineral content).

Three different digestion methods were applied to all samples (70% of HNO_3, ashing, and aqua regia) in order to measure the concentration of elements. From the results of the three different digestion methods **Figure 12** and **13,** the result showed that across all cities irrespective of the element determined the dry ashing method gave the highest values (mg/kg) for each element follwed by aqua regia and Nitric acid digestion method. The result (**Figure 12** and **13**) also showed that all elements determined and across all cites, there was no significant difference between the three different digestion methods except for Ca. Where the dry Ashing and Aqua regia method with the highest values were signficantly different (paired t-test, $p < 0.05$) to the nitric acid method which gave the lowest values across all sites. While the dry ashing and aqua regia method on the other hand, showed no signficant difference ($p > 0.05$) to each other **(Figure 12** and **13).** The data shows that there was a highly significant difference between the two different digestions method (ashing and nitric acid) *p*-value = 0.0093 for Ca. In addition, *p* value between aqua regia and nitric acid was siginficantly different ($p = 0.019$). Howevere, there was no significant difference between ashing and aqua regia.

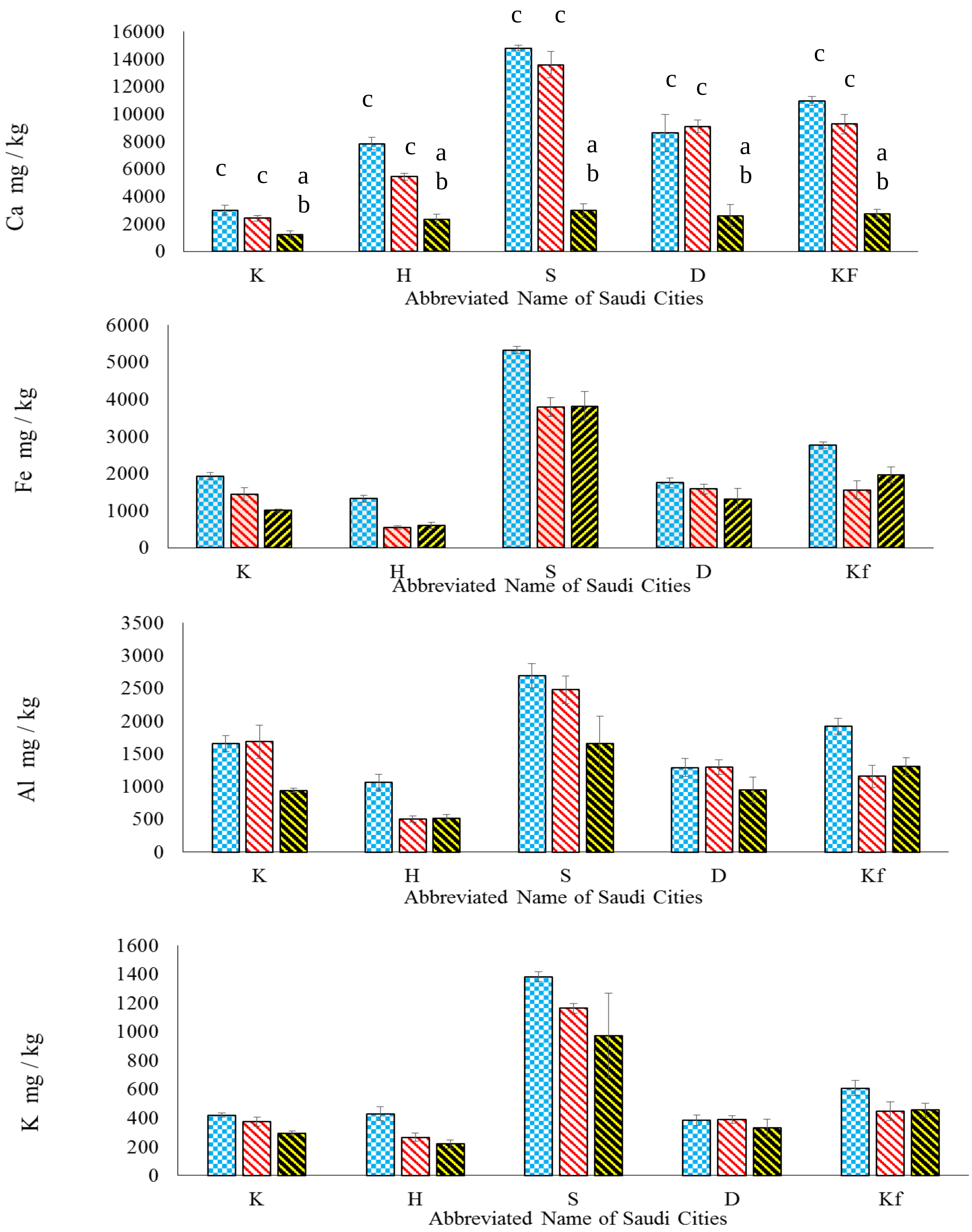


Figure 12 Comparison between three different digestion methods using ashing (blue), aqua regia (red) and HNO_3 (yellow with black) for five sand samples for the elements Ca, Fe, Al and K. (three replicates, error bars show one standard deviation). Lettering a, b and c refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with “a” when compared to the ashing, “b” when compared to aqua regia and “c” when compared to HNO_3 of digestion methods.

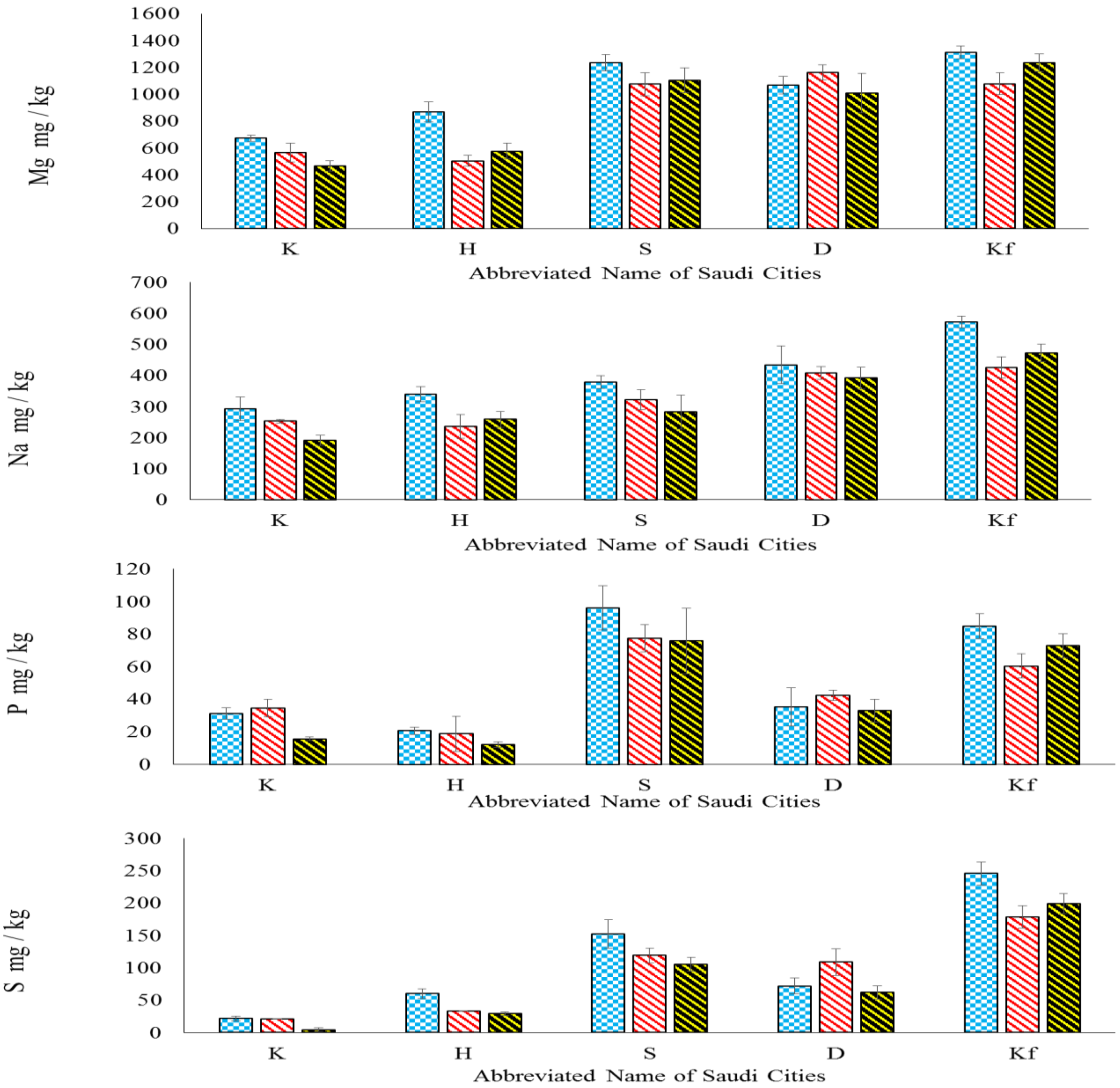


Figure 13 Comparison between three different digestion methods using ashing (blue), aqua regia (red) and HNO_3 (yellow with black) for five sand samples for the elements Mg, Na, P and S. (three replicates, error bars show one standard deviation). Lettering a, b and c refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with “a” when compared to the ashing, “b” when compared to aqua regia and “c” when compared to HNO_3 of digestion methods.

T-test of all the mentioned elements (Ca, Fe, Al, K Mg, Na, P and S) was conducted for the three digestion methods of each Saudi city (**Figure 14** and **15**). The results showed that there was a significasnt difference ($p < 0.05$) between the three methods of digestion (ashing, aqua regia and nitric acid) for each Saudi sand sample for most of the elements determined. All apart from sand sample Dammam (D) which showed no sigficanant differemce ($p > 0.05$) for Fe, K, Mg, P, Na and S.

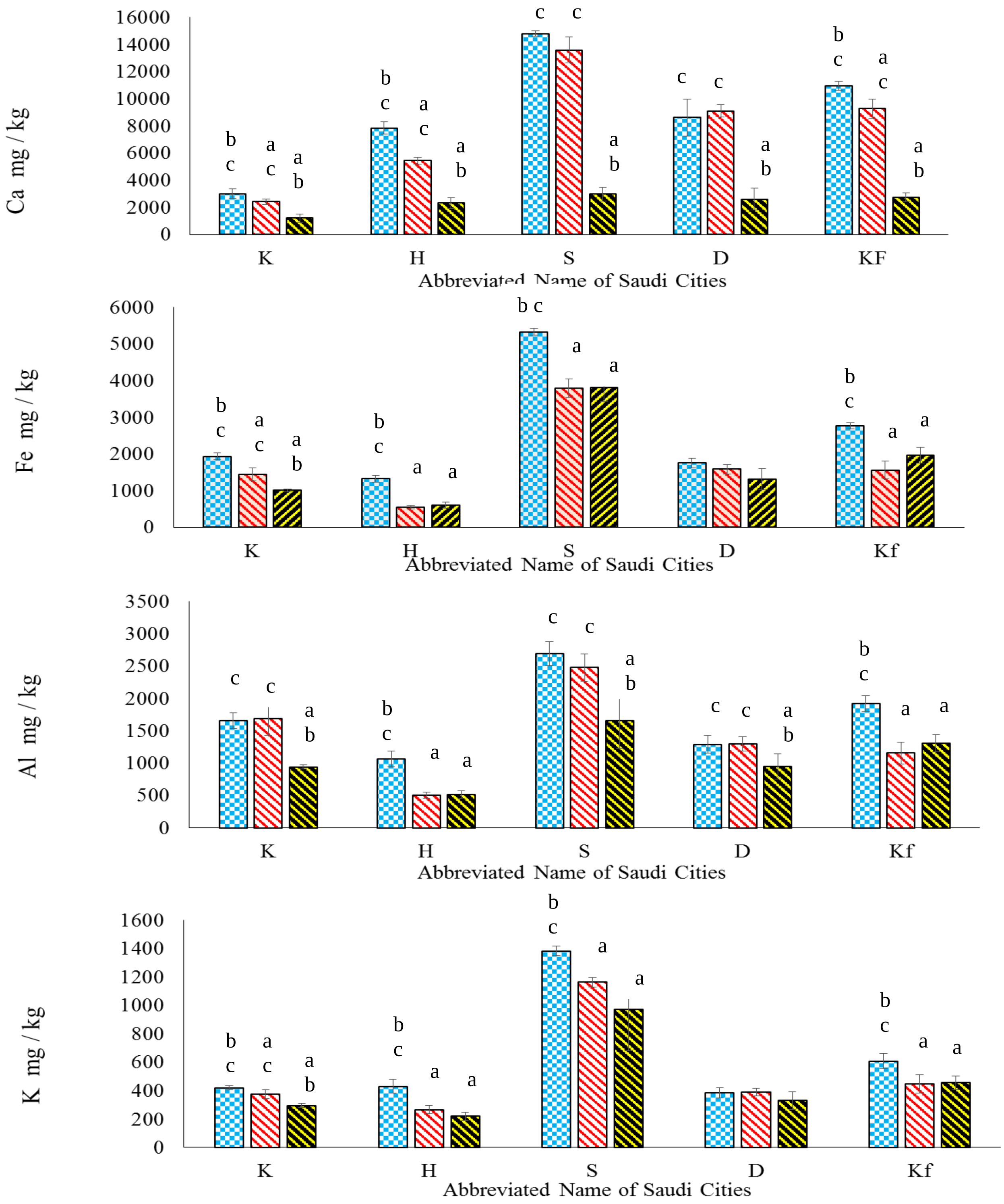


Figure 14 Comparison between three different digestion methods using ashing (blue), aqua regia (red) and HNO_3 (yellow with black) for five sand samples for the elements Ca, Fe, Al and K. (three replicates, error bars show one standard deviation). Lettering a, b and c refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with "a" when compared to the ashing, "b" when compared to aqua regia and "c" when compared to HNO_3 of digestion methods.

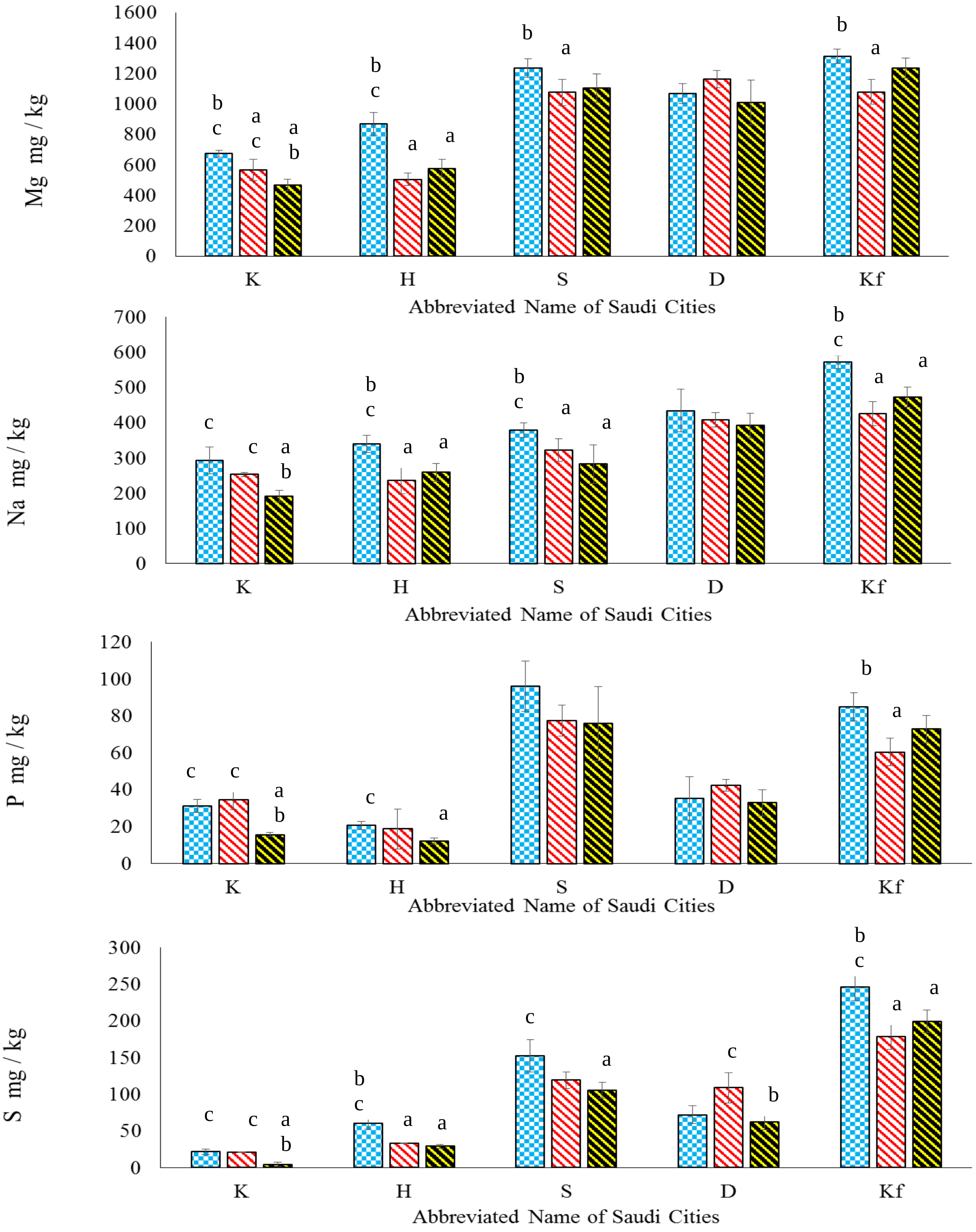


Figure 15 Comparison between three different digestion methods using ashing (blue), aqua regia (red) and HNO_3 (yellow with black) for five sand samples for the elements Mg, Na, P and S. (three replicates, error bars show one standard deviation). Lettering a, b and c refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with “a” when compared to the ashing, “b” when compared to aqua regia and “c” when compared to HNO_3 of digestion methods.

The results for the ashing method is shown in **table 9,** the results showed that there was a wide range in element concentrations in the sand samples. It was found that Ca, Fe, Al, Mg, K, and then Na had the highest average concentrations in all sand samples. Ca in sand samples ranged from 1310 mg/kg to 14823 mg/kg with an average of 5543 mg/kg. Fe ranged from 134 mg/kg to 6839 mg/kg with an average of 2590 mg/kg. In addition, Al ranged from 1065 mg/kg to 4575 mg/kg with an average of 1963 mg/kg. Mg ranged from 166 mg/kg to 2007 mg/kg with an average of 987 mg/kg.

Table 9 Total concentrations of some elements by ashing method reported as an average value of three replicate measurements ± standard deviation and expressed in mg/kg.

Element **Sample**	**Ca Mg/kg**	**Fe Mg/kg**	**Al Mg/kg**	**Mg Mg/kg**	**K Mg/kg**	**Na Mg/kg**
D	8625 ± 609	1636 ± 129	1334 ± 137	1064 ± 65	382 ± 35	434 ± 20
K	3013 ± 162	1940 ± 100	1733 ± 123	690 ± 24	421 ± 15	293 ±3 8
KF	10943 ± 329	2773 ± 81	1949 ± 123	1313 ± 48	608 ± 54	573 ± 19
H	7809 ± 377	1325 ± 91	1065 ± 122	869 ± 24	430 ± 47	340 ± 25
S	14823 ± 48	5385 ± 89	2692 ± 186	1236 ± 23	1386 ± 33	379 ± 20
Q	9563 ± 340	1533 ± 92	1155 ± 42	1349 ± 67	382 ± 30	335 ± 26
KO	9661 ± 177	1384 ± 15	1075 ± 46	1459 ± 46	355 ± 13	357 ± 13
R	6557 ± 52	1813 ± 89	1641 ± 44	563 ± 18	317 ± 7	287 ± 12
AN	1310 ± 18	134 ± 7	1742 ± 88	166 ± 7	55 ± 17	301 ± 8
B	1543 ± 77	2521 ± 44	2125 ± 76	908 ± 29	498 ± 23	270 ± 24
J	2333 ± 72	6839 ± 235	4575 ± 121	1754 ± 31	1175 ± 19	869 ± 35
HS	1471 ± 218	2900 ± 91	2000 ± 45	2007 ± 35	814 ±20	637 ± 11
Maximum	14823	6839	4575	2007	1386	869
Average	5543	2590	1963	987	545	479
Minimum	1310	134	1065	166	55	270

It was noticed that the concentration of each element varied across all sand samples. It was found that the major elements measured in the sand samples (Ca, Fa, Mg, Al, K, and Na) were the highest concentration (**Appendix 3**). Ca was the highest concentration in all samples. The concentration of Ca was highest in Sulayyil (14823 mg/kg ± 48) followed by Khafji, 10943 mg/kg ± 329, then followed by Khobar, 9661 mg/kg ± 177, followed by Qatif, 9563 mg/kg ±

340. It is found that the concentration of Ca in Riyadh to be 6557 mg/kg ± 52, while found in Hufuf as 7809 mg/kg ± 377. The lowest concentration of Ca in sand samples found to be 1310 ± 18 mg/kg in sample Unaizah.

Iron was the second most abundant element in most of the sand samples. In sample Sulayyil, the concentration of Fe was the highest at 5385 ± 89 mg/kg, whereas, the lowest concentration was found to be 134 ± 7 mg/kg in Unaizah. However, sample Buraidah had the highest concentration in Fe, 2521 mg/kg ± 44 rather than Ca. Therefore, AL was the second highest elemental concentration at 2125 ± 76 mg/kg followed by Ca at 1543 mg/kg ± 77. The samples, which were collected from Riyadh, Hufuf, Qatif, Sulayyil, Karj, Khafji, Buraidah, Unaizah, and Dammam, had Fe as the second highest concentration apart from Khobar, which had Mg, 1459 ± 46 mg/kg, as the second highest concentration. In addition, sample Buraidah had Fe as the highest concentration at 2521 mg/kg ± 44; and Al as the second highest concentration in elemental analysis to be 2125 ± 76 mg/kg.

2.3.3 Total petroleum Hydrocarbons Determination.

The total petroleum hydrocarbons (aliphatic and aromatic) in crude oil and diesel samples were determined using the GC-MS. The aliphatic and aromatic were fractionated using chromatographic column separation method using silica gel. A standard of aliphatic and aromatic compounds were used to develop the chromatographic column procedure (see section **2.3.3.1 - 2.3.3.2)**. The percentage recovery of both aliphatic and aromatic were determined.

2.3.3.1 Aliphatic Hydrocarbons Standard.

A certified alkane standard mixture of 34 aliphatic hydrocarbons from C_7 to C_{40} was purchased from Sigma at a concentration of 1000 ppm (mg/L) in Hexane. Using the same GC-MS method (method 3+1), the thirty-five compounds of aliphatic standard was run (**Figure 16**). The molecular weight, retention index (RI), and retention time for each peak and compound was calculated and is shown in **Appendix 4**.

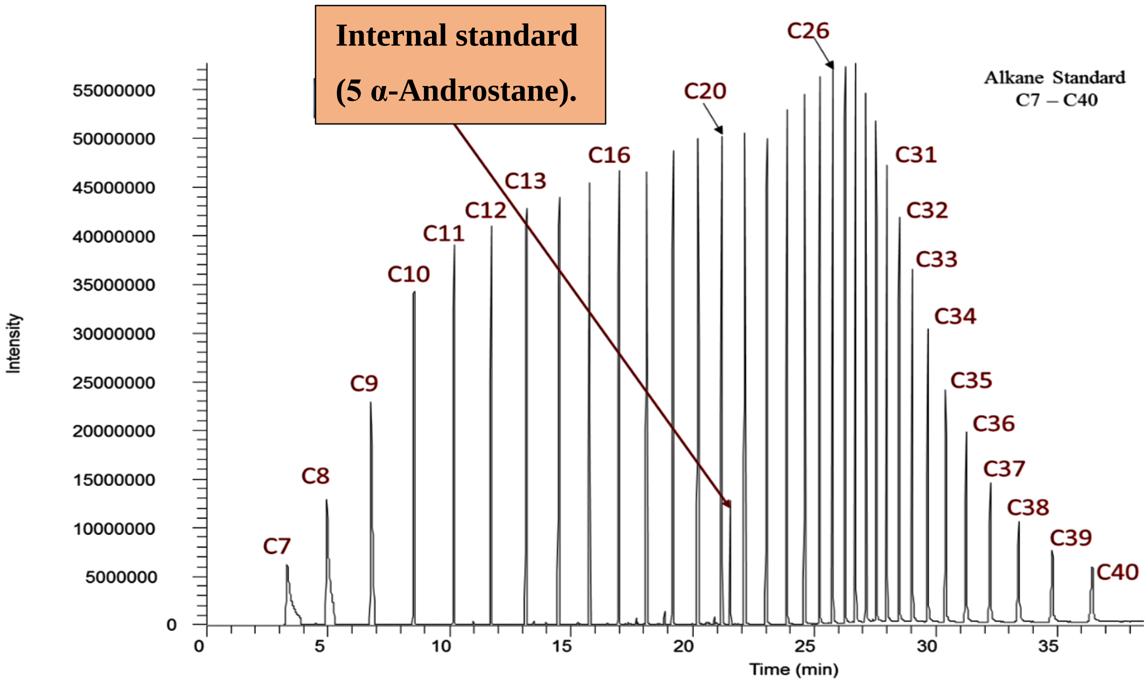


Figure 16 GC-MS chromatogram of alkane standard C_7 – C_{40} with internal standard (5 α-Androstane).

2.3.3.2 Aromatic Hydrocarbon Standards.

Using the GC-MS method and instrument setting stated in **table 7–8 (page 33-34)**, the sixteen compound aromatic standard was run (**Figure 17**). The molecular weight and retention times for each peak and compound was calculated and shown in the following table (**Table 10**).

Table 10 Aromatic hydrocarbons standard.

Compound	Retention Time (min)	MW g/mol	Compound	Retention Time (min)	MW g/mol
Naphthalene	13.43	128.0	Pyrene	24.42	202.12
2-Methylnaphthalene	15.71	142.0	Benzo(a)Anthracene	24.79	228.21
Acenaphthylene	18.85	152.0	Chrysene	26.78	228.20
Acenaphthene	19.12	154.2	Benzo(k)Fluoranthene	26.86	252.27
Fluorene	20.35	166.10	Benzo(b)Fluoranthene	28.51	252.27
Phenanthrene	22.29	178.03	Indeno(1,2,3-cd)Pyrene	28.58	276.34
Anthracene	22.38	178.07	Benzo(g,h,i) Perylene	31.66	276.33
Fluoranthene	24.03	202.12	Dibenzo (a,h)Anthracene	32.31	278.35

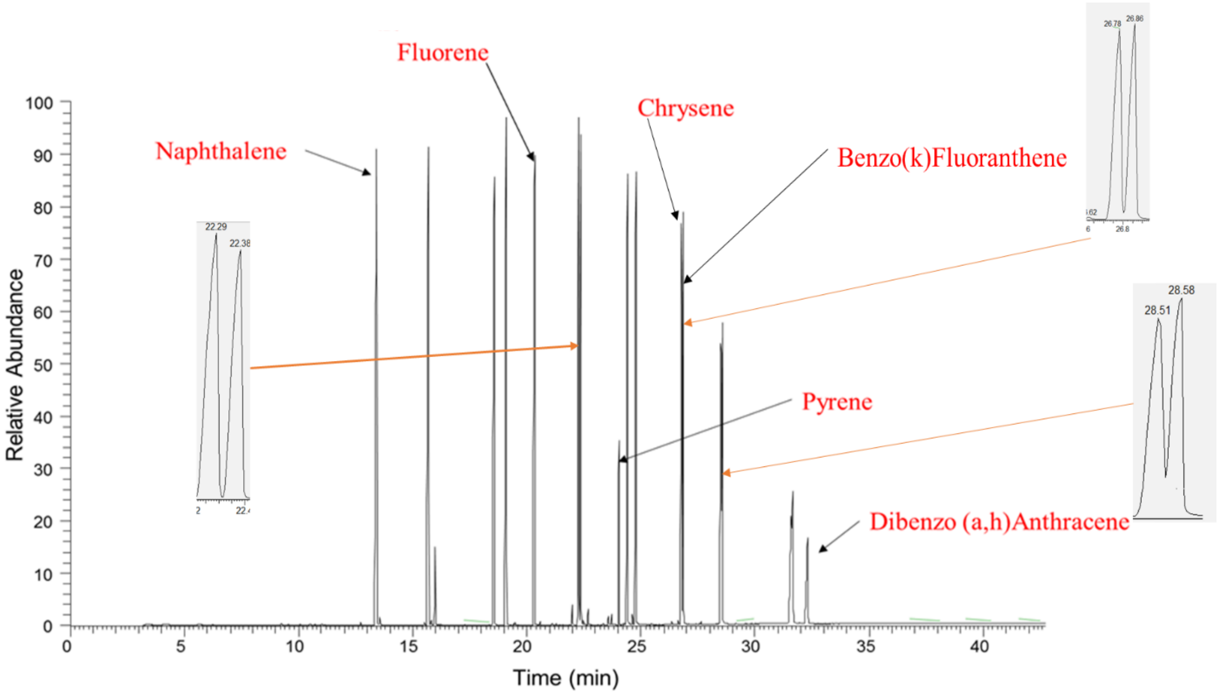


Figure 17 GC-MS chromatogram for PAHs standards.

2.3.4 Fractionation of Hydrocarbons into Aliphatic and Aromatic Fractions Using Chromatographic Column Separation Method.

Hydrocarbon mixtures can be separated into aliphatic and aromatic fractions using activated silica gel. **Figure 18** shows the GC-MS chromatograms for the aliphatic, aromatic and combined fractions. Chromatogram A is the aliphatic fraction (F_1) obtained using hexane as a solvent. This is recognisable because of the even spacing for the long chain hydrocarbons, as shown for the alkane standard solution above. The aliphatic range is from C_7 to C_{31} for the crude oil sample. . Chromatogram B is the aromatic hydrocarbon fraction (F_2) obtained using DCM-Hexane (50-50%) as a solvent. Chromatogram B differs from A in the disappearance of the equally spaced alkanes and the increased area of the remaining peaks. Comparison with the PAH standard (**Figure 18**) only resulted in three identifications, based on comparison with the standard and confirmation using the MS, which were naphthalene, 2-methylnaphthalen, and phenanthrene. This confirms that the separation of the aliphatic from the aromatic fraction was successful. Chromatogram C was a combination of F_1 and F_2 in order to get TPHs (F_3) and is shown in chromatogram C. Chromatogram D (F_4) was a hexane fraction obtained after the DCM-Hexane (50-50%) fraction in order to check that there were no hydrocarbons remaining on the silica column. Chromatogram D shows this hexane check solution (F_4) and illustrates that the volumes to obtain the aliphatic and aromatic fractions were appropriate.

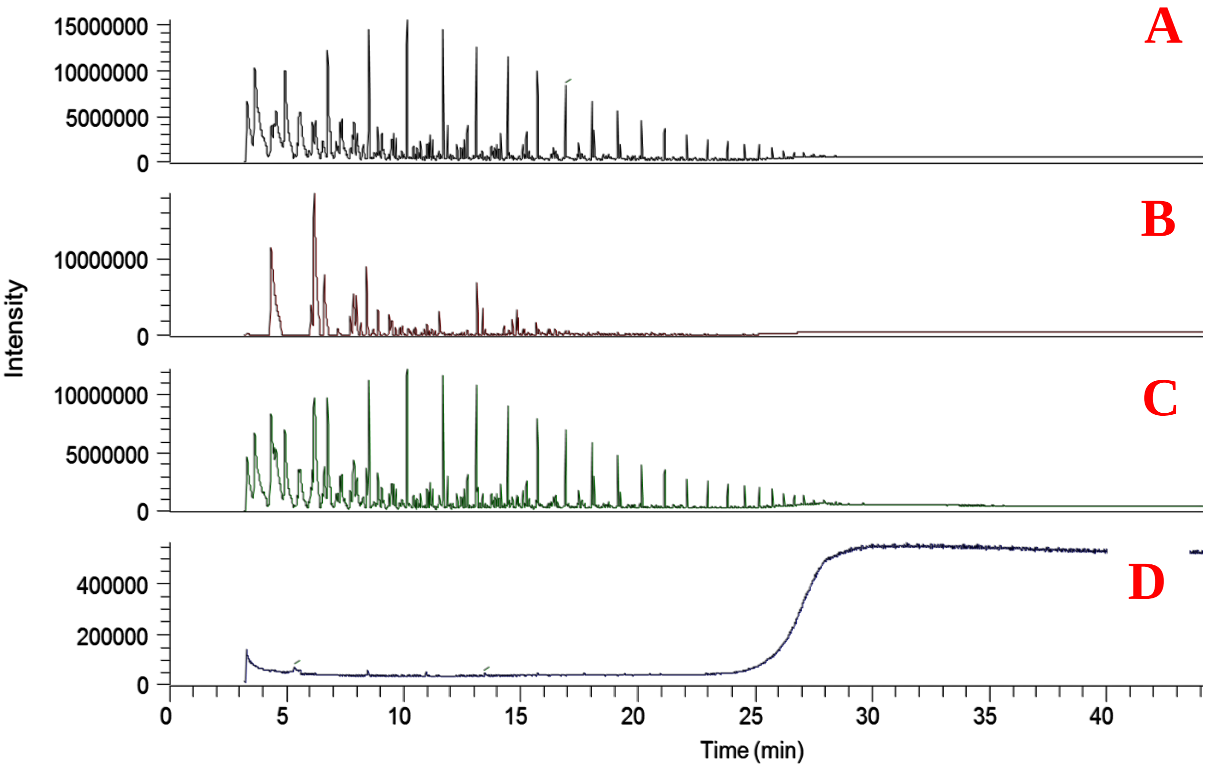


Figure 18 Chromatogram of crude oil fractions using chromatographic separation method. A, B and C show the chromatogram of the aliphatic (F_1), aromatic (F_2) and combined TPH (F_3) fractions. D shows the chromatogram of the wash fraction.

In addition, this fractionation method was applied for the diesel sample. **Figure 19** shows, the GC-MS chromatograms for aliphatic, aromatic and combined fractions for diesel. Chromatogram A is aliphatic fraction (F_1) obtained using hexane as solvent. This is recognisable because of the even spacing for the long chain hydrocarbons, as shown for the alkane standard solution above. The range is from C_9 to C_{27} for the diesel sample. Chromatogram B is the aromatic fraction (F_2), DCM-Hexane (50-50%) was used as a solvent in order to extract aromatic hydrocarbons. Chromatogram B differs from A in the disappearance of the equally spaced alkanes and the increase in area of the remaining peaks. Comparison with the PAH standard (**Figure 17 (page 46)**) there was no peak comparable to the PAHs standard. F_3 was a combination of both F1 and F2 in order to get TPHs and is shown in chromatogram C.

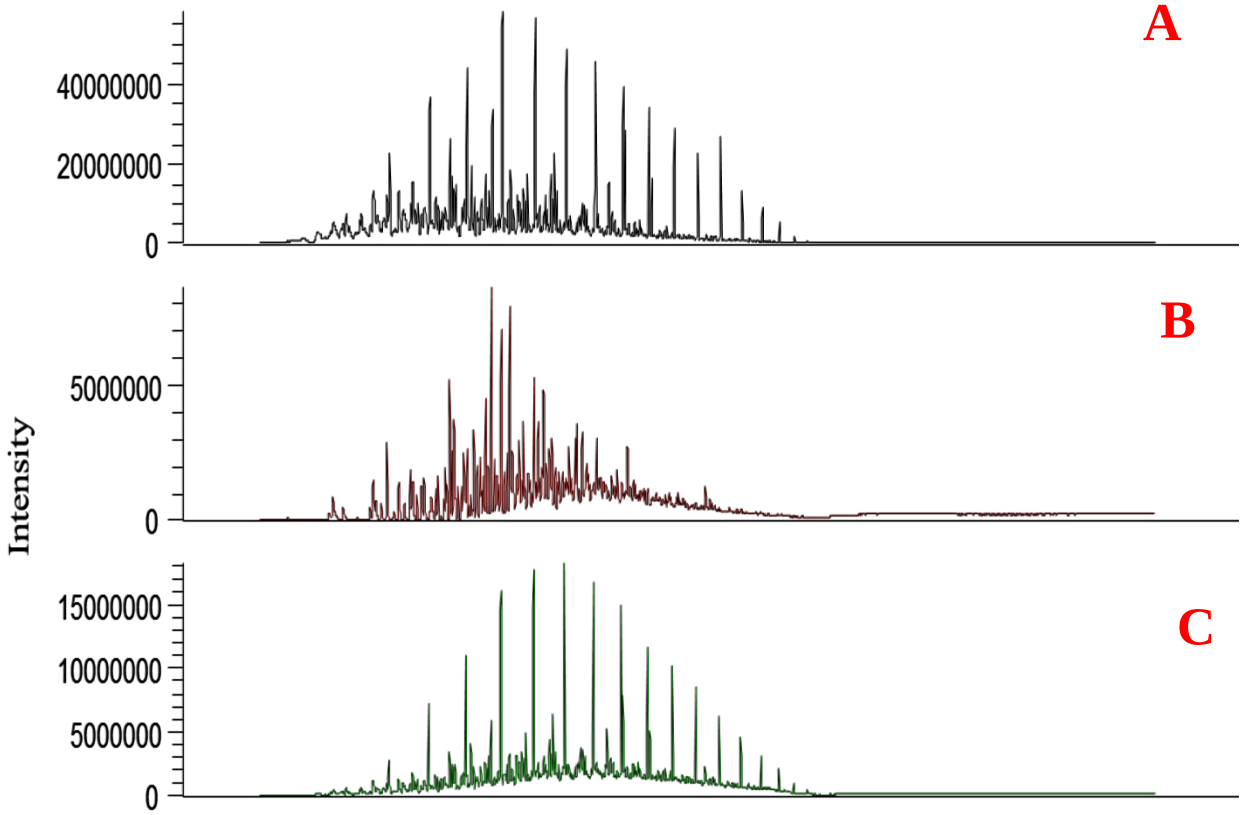


Figure 19 Diesel fractionation of commercial diesel using chromatographic method. A, B and C show the chromatograms of the aliphatic (F_1), aromatic (F_2) and combined TPH (F_3) fractions.

Therefore, the above figure shows a clear separation between aliphatic and aromatic compounds in TPHs.

2.3.5 Determination of Percentage of Aliphatic and Aromatics in Crude Oil and Diesel.

The result for the chromatographic separation of crude oil in to aliphatic(F_1) and aromatic (F_2) fractions using two different weights (25 mg and 50 mg) of crude oil is shown in **table 11**.The result shows that for the 25 mg crude oil mixture, the TPH in fractions (F_1 and F_2) was 23.18 mg (90 % recovery). The crude oil mixture was found to contain 81 % and 19 %

of aliphatic (F_1) and aromatic compounds (F_2) respectively. For comparative purposes, the experiment was repeated with a crude oil mixture of twice the concentration (50 mg) and the result show that about 80 % of both aliphatic (F_1) and aromatic (F_2) compounds were recovered. The recovered fractions contained 79 % (F_1) and 21 % (F_2), which was comparable to the 25 mg crude oil mixture (**Table 11**).

Table 11 Determination of percentage of aliphatic and aromatics in diesel obtained through chromatographic separation method. Average of three replicates.

Quantity of diesel (mg)	F1 +F2 (mg) + RSD (%)	Compounds retained on column (mg)	Recovery (%)	F1 (%)	F2 (%)
25.6mg	**23.18 ± 1.9**	**2.42**	**90**	**81**	**19**
50mg	**39.79 ± 2.8**	**10.21**	**80**	**79**	**21**

The result for the chromatographic separation of crude oil in to aliphatic(F_1) and aromatic (F_2) fractions using two different weights (25 mg and 50 mg) of crude oil is shown in **table 12**.The result shows that for the 25 mg crude oil mixture, the TPH in fractions (F_1 and F_2) was 7.06 mg (28.1% recovery). The crude oil mixture was found to contain 68.6 % and 31.3 % of aliphatic (F_1) and aromatic compounds (F_2) respectively. For comparative purposes, the experiment was repeated with a crude oil mixture of twice the concentration (50 mg) and the result show that about 30.4 % of both aliphatic (F_1) and aromatic (F_2) compounds were recovered. The recovered fractions contained 69.5% (F_1) and 30.4 % (F_2), which was comparable to the 25 mg crude oil mixture (**Table 12**).

Table 12 Determination of percentage of aliphatic and aromatics in crude oil. Average of three replicates.

Quantity of crude oil (mg)	F1 +F2 (mg)) + RSD (%)	Compounds retained on column (mg)	Recovery (%)	F1 (%)	F2 (%)
25 mg	**7.06 ± 0.02**	**17.9**	**28.1**	**68.6**	**31.3**
50 mg	**15.2 ± 0.01**	**34.8**	**30.4**	**69.5**	**30.4**

70% of crude oil contains volatile hydrocarbons (under C_{12}), polar compounds retained on column, and possibly asphaltenes (hexane insoluble constituents). However, the recovery percentage of crude oil was approximately 30% of the original volume, which is low. This might be because some compounds are volatile and some of the crude oil hydrocarbons would remain in the column stuck to the silica gel. Therefore, after getting the percentage, a GC-MS for the crude oil fractionation was ran in order to find out if the hydrocarbons under C_{12} disappeared from the column or not. The recovery percentage of crude oil was compared to both crude oil fractionation and original crude oil sample chromatograms (**Figure 20**). The result found that the first peaks in crude oil fractionation volatised, which justifies the lower recovery percentages of crude oil. Therefore, using the gravimetric method in crude oil recovery would not be an accurate method to recover high percentage of crude oil.

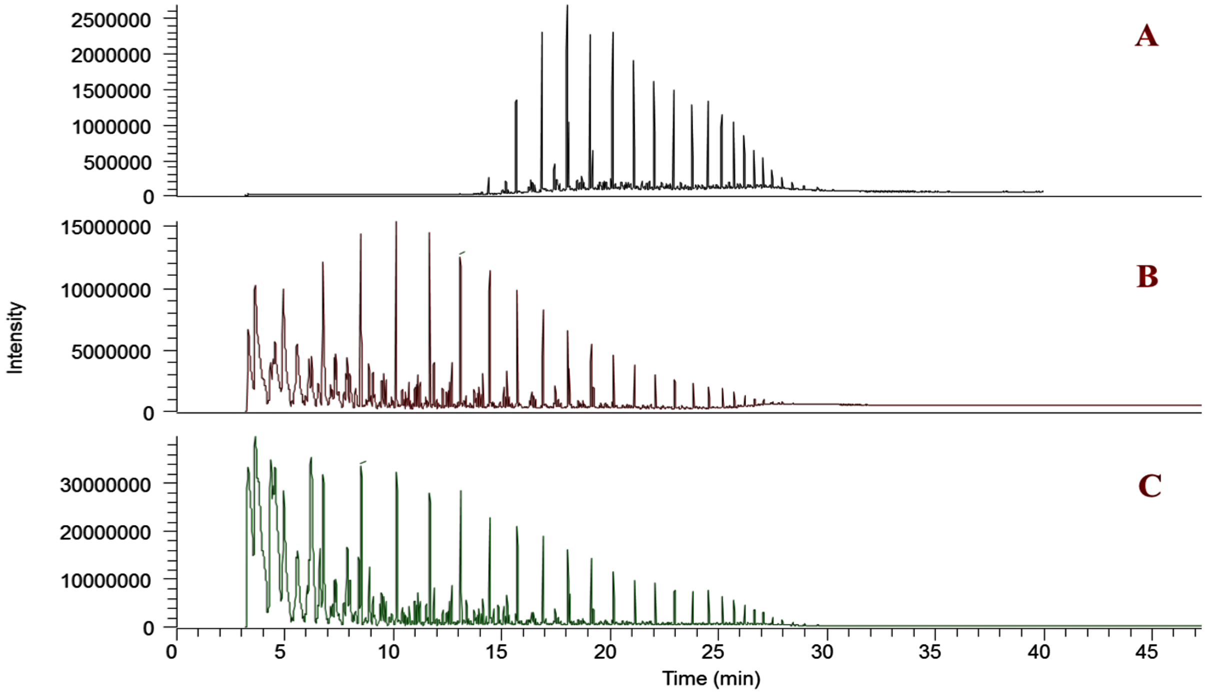


Figure 20 Comparison of crude oil sample after application of chromatographic separation method and sample reduced to dryness (A) with crude oil sample after application of chromatographic separation method and sample reduced to a few ml (B) and with unfractionated crude oil sample (C).

2.3.6 Determination of specific gravity in Hydrocarbons (Crude oil and Diesel).

Specific gravity is an important parameter to measure the quality of crude oils. Low specific gravity indicates good quality of crude oil, having lighter fractions, and vice versa. The specific gravity of the crude oil gives a rough measure of the amount of lighter hydrocarbons present. Crude oil samples can be classified as light, medium or heavy according to their API gravity. API is a specific gravity scale developed by the American Petroleum Institute for measuring the relative density of various petroleum liquids using the following formula.

$$\mathrm{API} = \frac{141.5}{\mathrm{SG}} - 131.5$$

Where SG is Specific Gravity.

The calculated specific gravity of crude oil was found to be 0.7343 ± 0.0155. At a temperature of 18 ^{o}C, API gravity was found to be 61.20 for crude oil. It stated that light crude API is greater than 31.1 and heavy crude API is less than 22.3. Between 22.3 and 31.1, the crude oil is classified as medium. Based on the results the crude oil in this project found to be as light crude oil according to the API gravity.

Regarding diesel fuel, it is stated that the specific gravity of diesel fuel is 0.832. In this project the specific gravity for diesel was found to be 0.8302 at 20 °C, which confirms the reported value of specific gravity is comparable. In order to make sure that the determination steps of specific gravity in crude oil were accurate, the specific gravity in the diesel sample conducted to be 0.8302 ± 0.007 at 20 °C. This confirms that the specific gravity of crude oil is accurate and correct according to the calculation of diesel specific gravity.

2.3.7 Aliphatic Compounds in the Sand Samples (Leaching Experiment).

GC-MS calibration standards were prepared to produce a calibration curve with a range of 50 – 400 ppm using 50 ppm of internal standard (5 -α Androstane). The distribution of aliphatic hydrocarbons was determined using peak area ratios (peak area / internal standard). Leaching experiments were applied for five samples. An example GC-MS chromatogram for sand sample Dammam (D) shown the water extractable hydrocarbon is given in the following **Figure 21**.

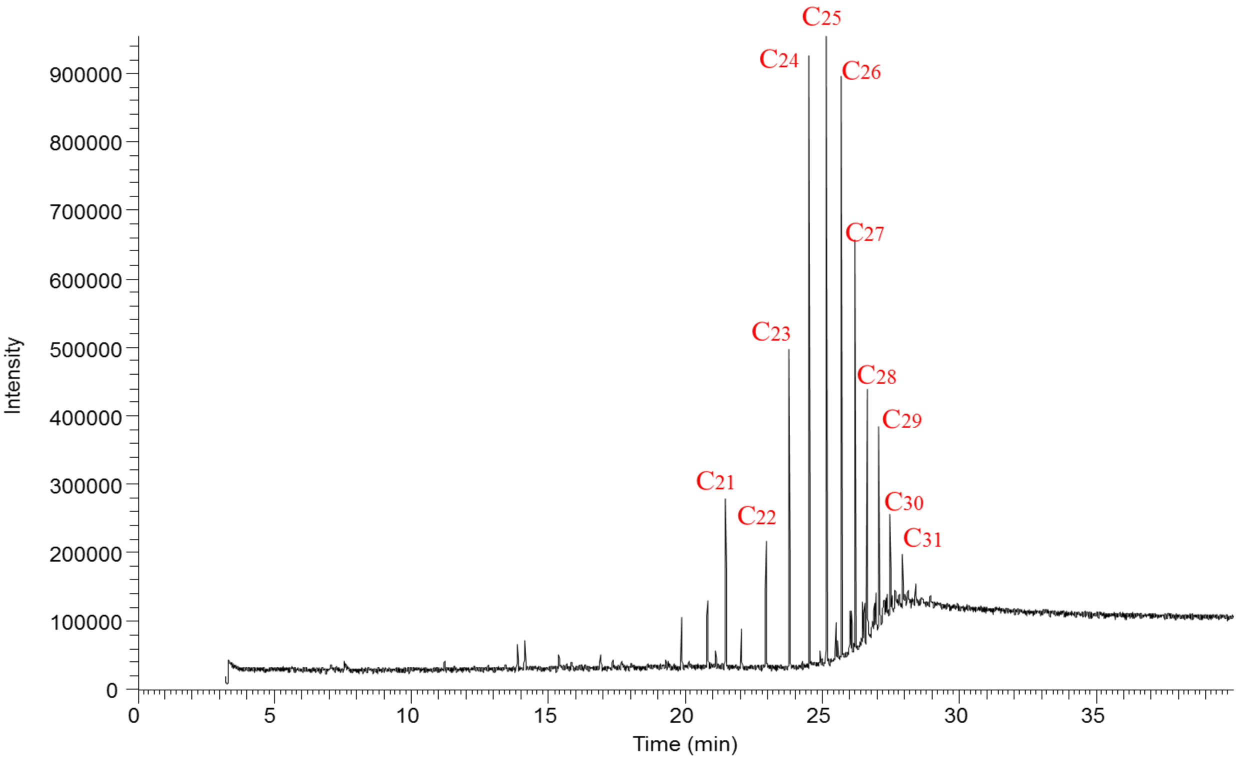


Figure 21 Example of GC-MS chromatogram for water extractable hydrocarbons of Saudi sand sample (D) using method and experimental setting in table 7 and 8.

The hydrocarbons concentration of Buraidah, Dammam, Riyadh, Hufuf and Sulayyil samples varied from C_{21} to C_{36} (**Figure 22**). The highest concentration of extractable aliphatic hydrocarbon (C_{29}) in Hufuf was found to be 1212.78 mg/kg while the lowest concentration was found in C_{36} (3.74 mg/kg). In addition, the highest concentration in Sulayyil was found in C_{29} to be 987.63 mg/kg while the lowest concentration found in C_{35} to be 22.70 mg/kg. Moreover, the highest concentration in Buraidah was found in C_{25} to be 249.88 mg/kg followed by C_{24} to be 240.04 mg/kg, while the lowest concentration was found in C_{31} to be 9.75 mg/kg. The highest concentration in Dammam found in C_{25} to be 354.04 mg/kg while, the lowest concentration was found in C_{21} to be 29.20 mg/kg. Finally, the highest concentration in Riyadh was found in C_{29} to be 114.34 mg/kg while, the lowest concentration was in C_{33} to be 8.03mg/kg.

Preliminary results suggest that after leaching with water (**Figure 22**), samples contained 16 aliphatic hydrocarbons (C_{21} – C_{36}). Sand samples Hufuf and Sulayyil contained higher concentrations of aliphatic hydrocarbons compared to other samples (Buraidah, Dammam, and Riyadh). Quantification of the aliphatic hydrocarbons in the sand samples using an internal standard method and integration based on area ratios showed that sample Riyadh contained the lowest concentration of water extractable aliphatic hydrocarbons (C_{21}- C_{36}). Samples Hufuf, Sulayyil and Riyadh, showed the same trend in terms of hydrocarbon concentration (C_{23} – C_{36}). Sample Dammam and sample Buraidah on the other hand, showed a different trend with C_{24} – C_{26} having the highest concentrations, while the others showed hydrocarbon concentrations from C_{27}- C_{31}. This suggest that sample Dammam and sample Buraidah likely contain more of lower hydrocarbons (C_{21}- C_{26}) compared to the others. This also suggests that the degradation of lower hydrocarbon in sand (Dammam and Buraidah) was likely occurring at a much slower rate compared to other samples. The sand texture could also have affected the extractability of the higher hydrocarbons in sample Dammam.

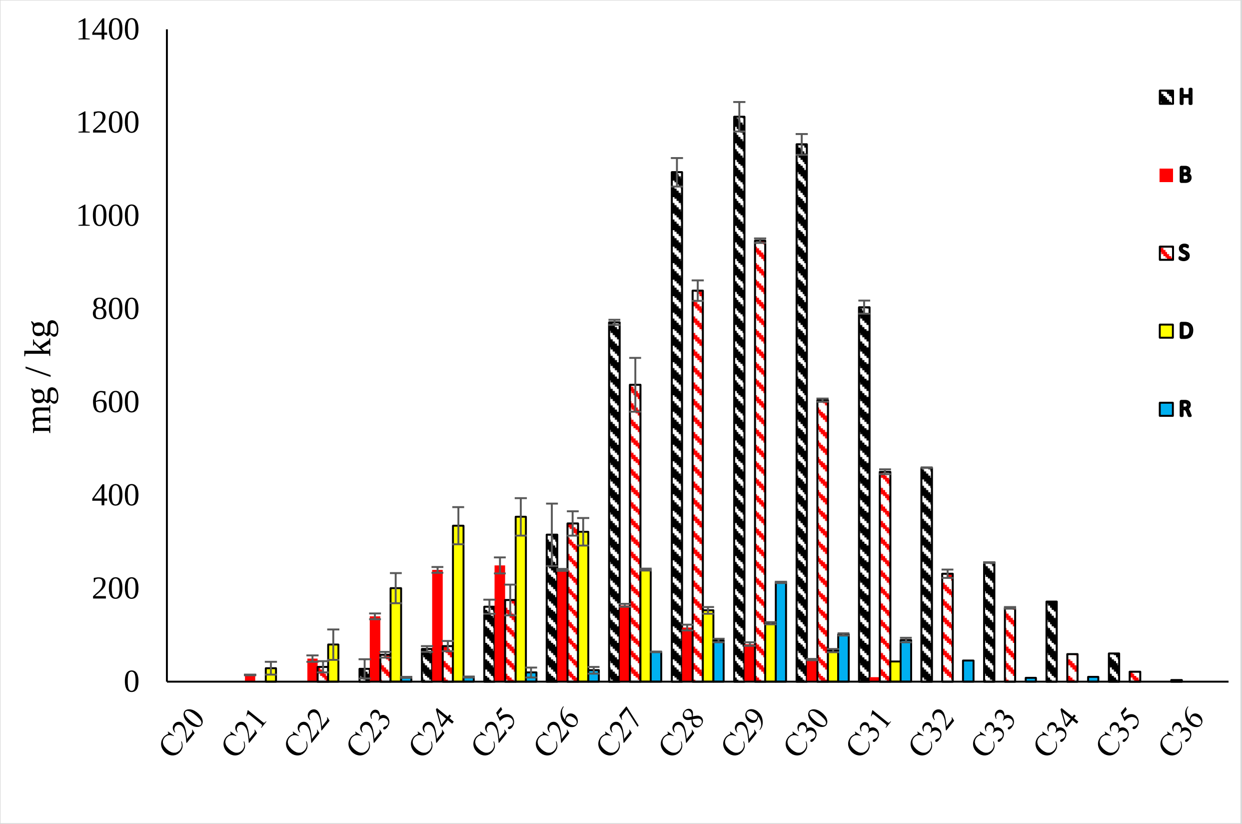


Figure 22 Distribution of water extractable aliphatic hydrocarbons in the Saudi sand Buraidah (B), Dammam (D), Riyadh (R), Hufuf (H) and Sulayyil (S) samples.

From the above figure, the total aliphatic hydrocarbons concentration for the five sand samples ranged from 586 mg/kg to 6651 mg/kg using the leaching experiment. The highest concentration of total aliphatic hydrocarbons was found in sample Hufuf at 6651 mg/kg. The total aliphatic hydrocarbon concentration in sand Sulayyil was found to be 1352 mg/kg. In addition, the total aliphatic hydrocarbon concentration in sand Dammam and Buraidah found was found to be 1950 mg/kg 1352 mg/kg, respectively, while the lowest concentration of total aliphatic hydrocarbons was found in sample Riyadh at 586 mg/kg.

2.3.8 Quantification of Extractable Aliphatic Compounds in the Sand Samples Using DCM Shaking for 24 hour.

A range of hydrocarbons was extracted from 14 sand samples using mechanical shaking for 24 hours (**Figure 23** and **24**). Some sand samples have a high percentage of hydrocarbons while others have a lower percentage of hydrocarbons (**Figure 25-28**).

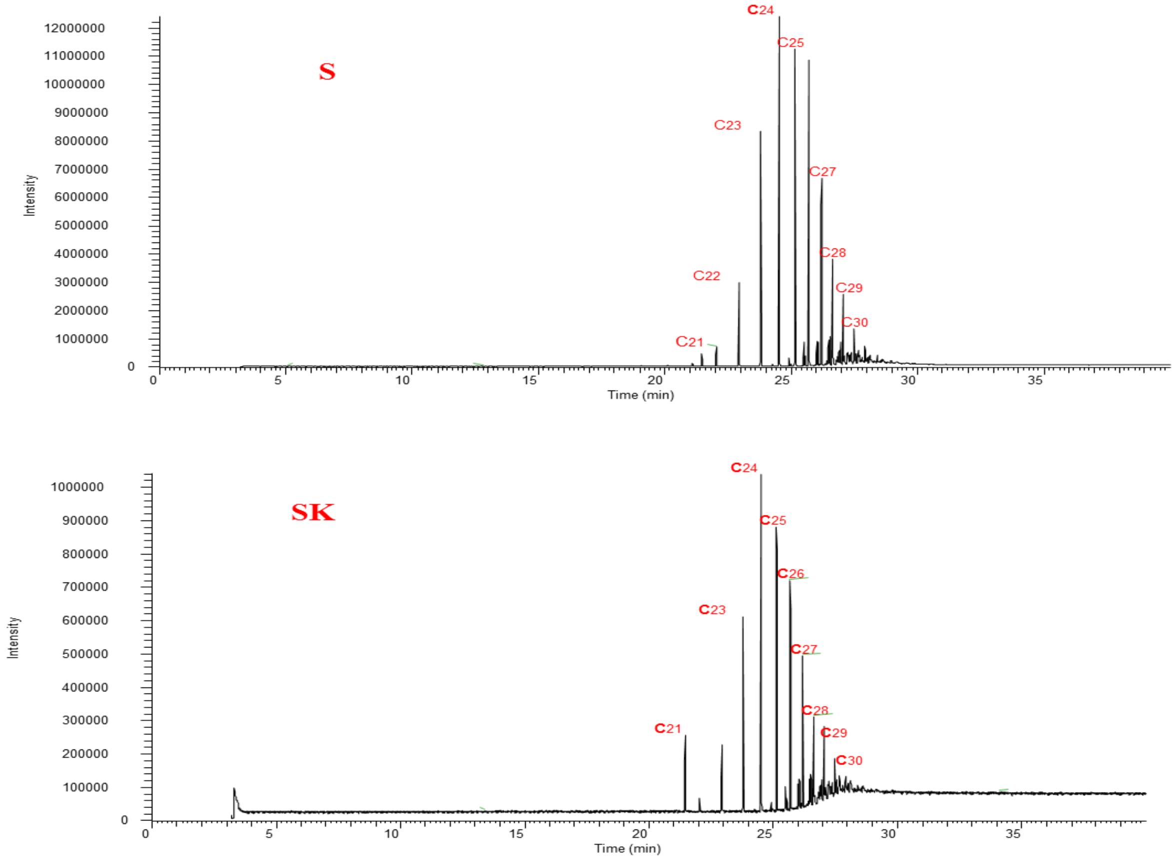


Figure 23 GC-MS chromatograms of Saudi sand samples Sulayyil (S) and Skaka (SK) showing the range of hydrocarbon peaks.

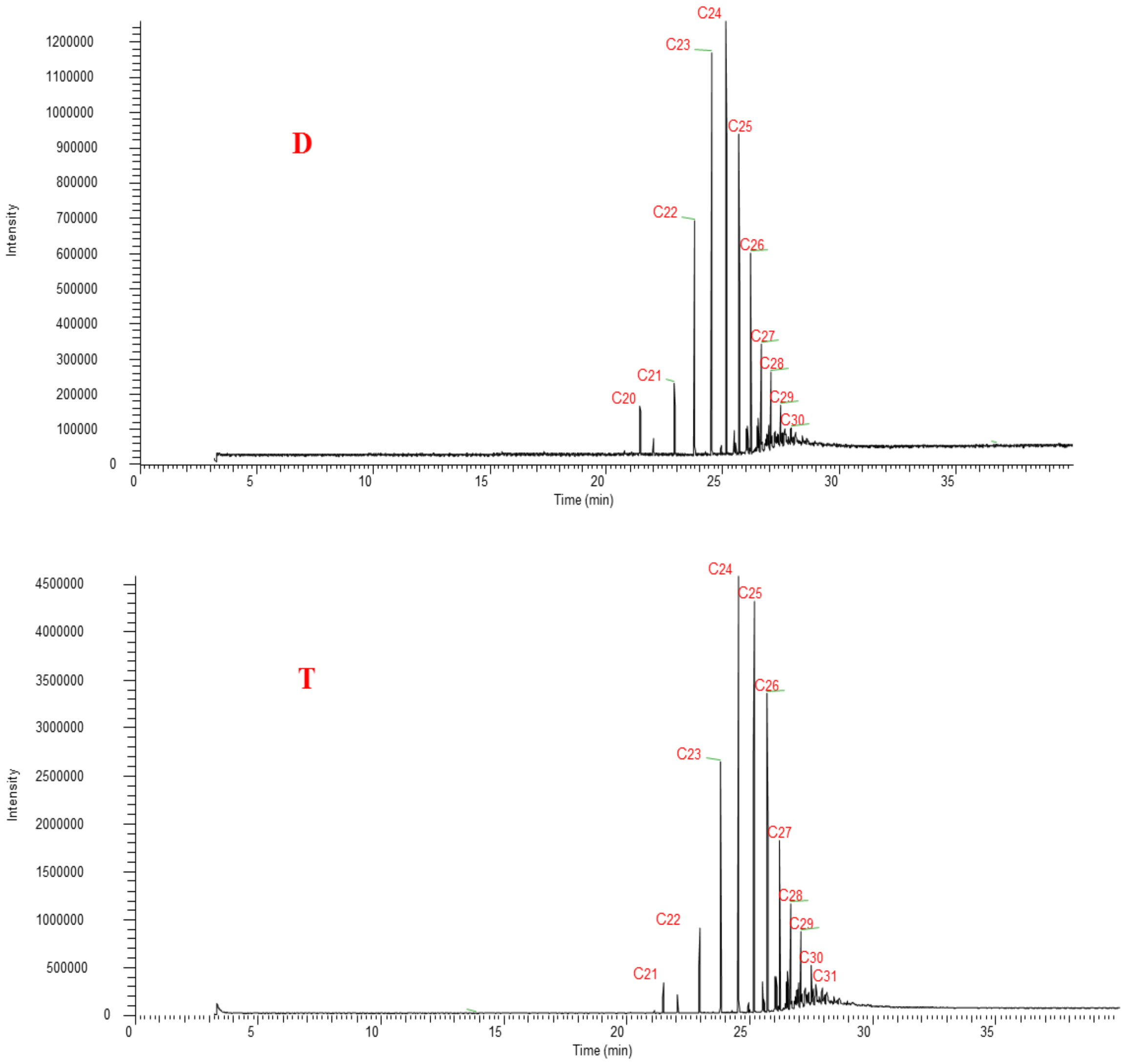


Figure 24 GC-MS chromatograms of Saudi sand samples Dammam (D) and Tabuk (T) showing the range of hydrocarbon peaks.

The total aliphatic hydrocarbons concentration (sum of C_{20} to C_{31}) for the 14 samples ranged from 234 mg/kg to 34708 mg/kg using mechanical shaking **(Figure 25 – 28)**. The total concentration of aliphatic hydrocarbons in sample Karj was found to be 34708 mg/kg. In sample Najran, the total concentration of aliphatic hydrocarbons was found to be 28008 mg/kg. In sample Khafji, the total concentration of aliphatic hydrocarbons was found to be 20995 mg/kg. In sample Sulayyil, the total concentration of aliphatic hydrocarbons was found to be 20347 mg/kg. While in sample Hufuf, Buraidah and Tabuk, the total concentration of aliphatic concentration was found to be 15285 mg/kg, 11911 mg/kg and 5935 mg/kg, respectively. In sample Dammam, Unaizah and Skaka, the total aliphatic concentration was found to be 3386 mg/kg, 2190 mg/kg and 1874 mg/kg, respectively. In sample Riyadh, Jeddah and Al-Hassy, the total aliphatic concentration was found to be 1414mg/kg, 618mg/kg and 483 mg/kg, respectively. The lowest concentration in total aliphatic hydrocarbons was found in sample Khobar at 234 mg/kg

It was found that the hydrocarbons from most of Saudi sand samples extracted aliphatic hydrocarbons from C_{20} to C_{31} using DCM mechanical shaking for 24 hours (**Figure 25-28**).

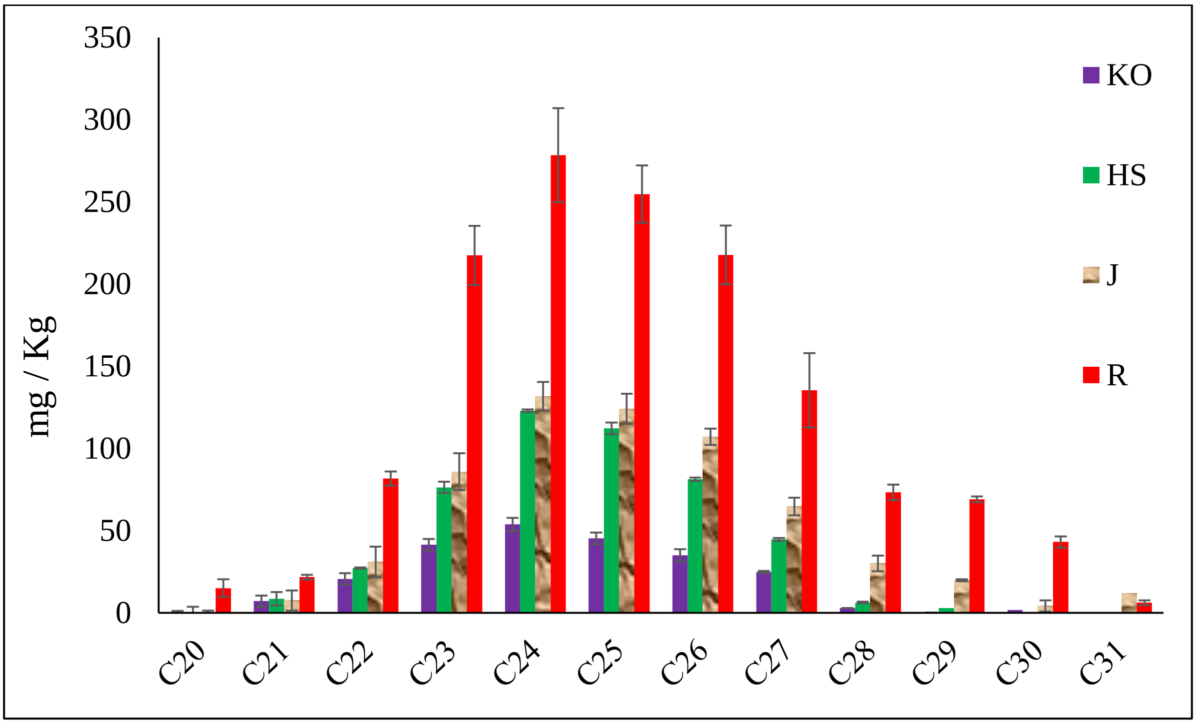


Figure 25 Distribution of aliphatic hydrocarbons by DCM 24 h shaking in Saudi sand samples, Khobar (KO), Al-Hassy (HS), Jeddah (J) and Riyadh (R), which had the lowest concentration of aliphatic hydrocarbons of all other Saudi sand samples.

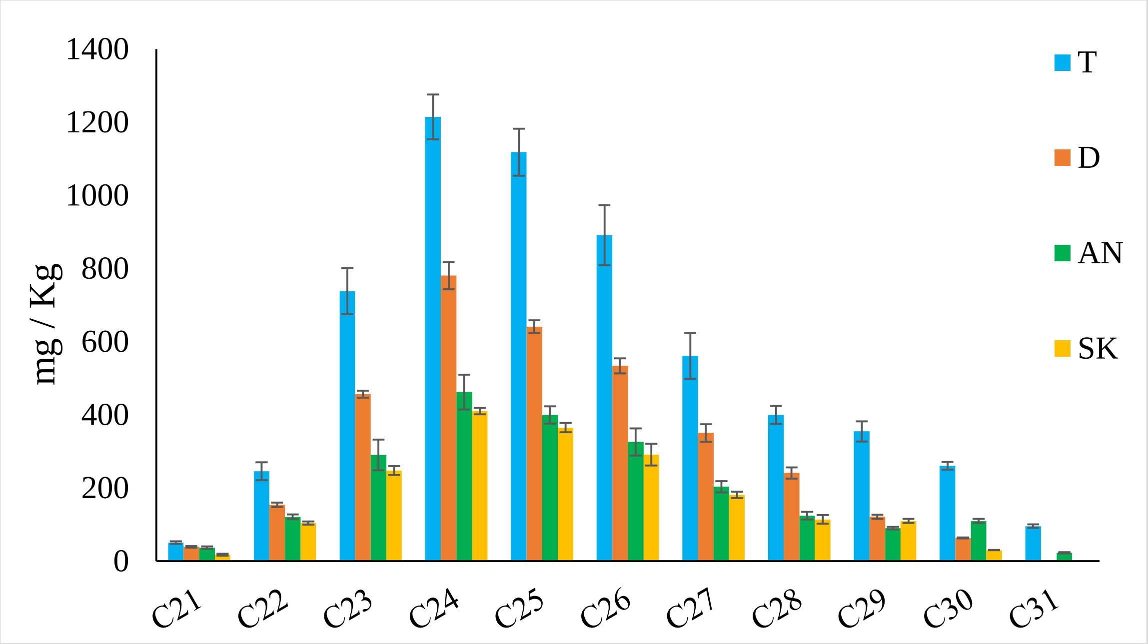


Figure 26 Distribution of aliphatic hydrocarbons by DCM 24 h shaking in Saudi sand samples, Tabuk (T), Dammam (D), Unaizah (AN) and Skaka (SK), which had the second lowest concentration of aliphatic hydrocarbons.

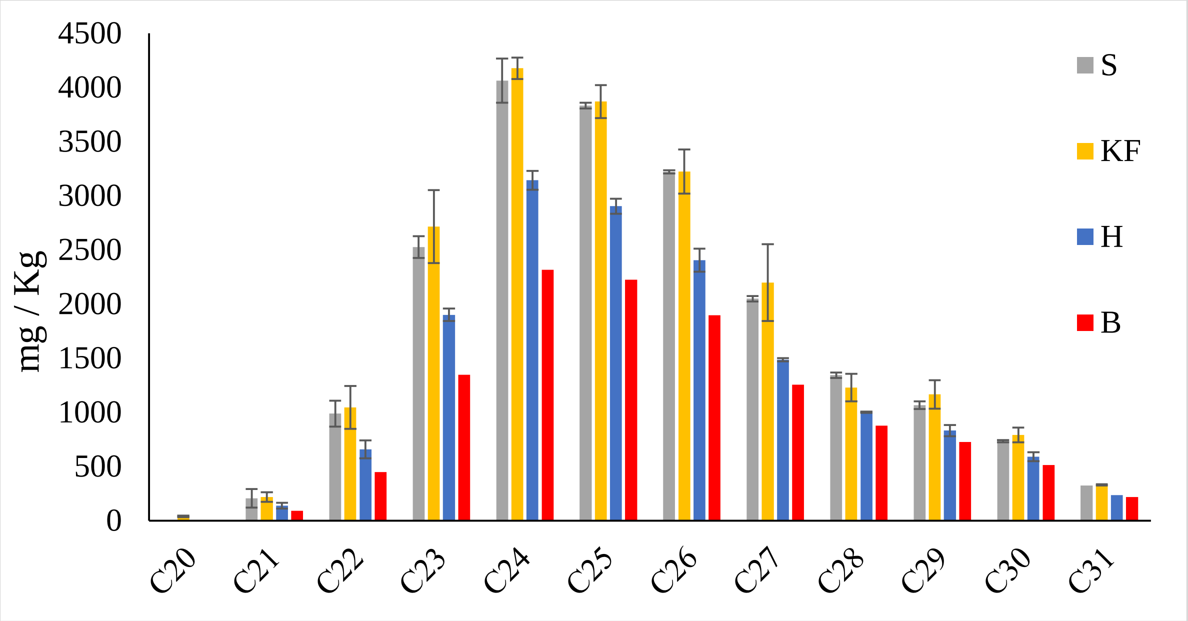


Figure 27 Distribution of aliphatic hydrocarbons by DCM 24 h shaking in Saudi sand samples, Sulayyil (S), Khafji (KF), Hufuf (H) and Buraidah (B), which had above average concentration of aliphatic hydrocarbons.

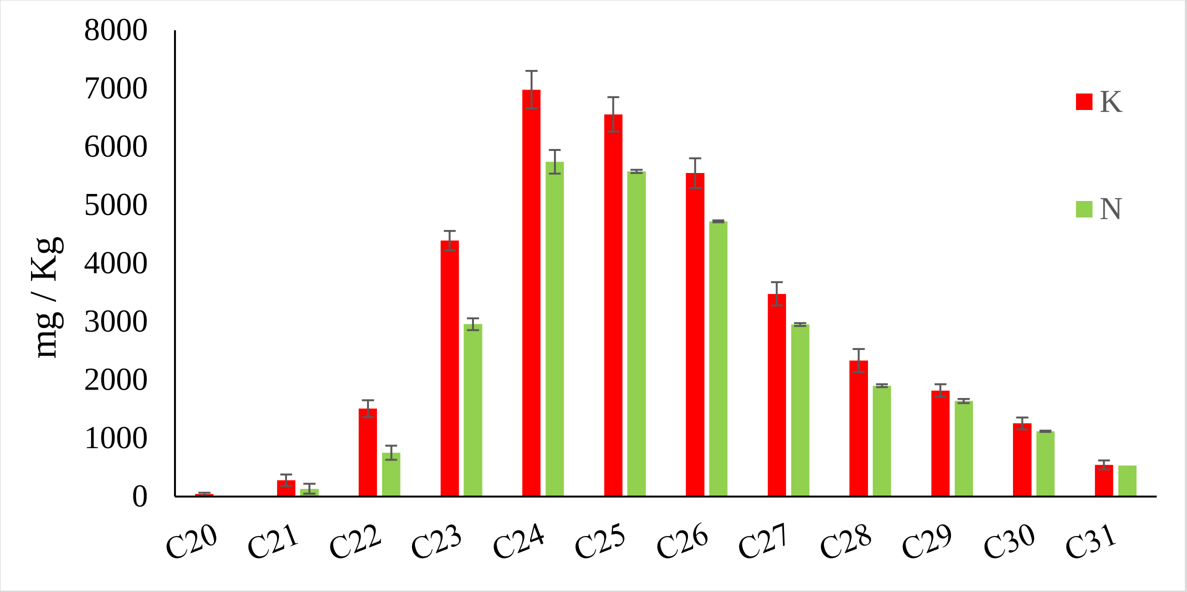


Figure 28 Distribution of aliphatic hydrocarbons by DCM 24 h shaking in Saudi sand samples, Karj (K) and Najran (N), which had the highest concentration of aliphatic hydrocarbons.

It was noticed that in all samples, the C_{24} was the highest concentration peak followed by C_{25}. While the lowest concentration peak found in most samples was C_{21} **(Table 13)**. Therefore, according to these results, the all samples have the same shape however, different quantities.

Table 13 Concentration (mg/kg) range of extractable aliphatic hydrocarbons in 14 cities in Saudi Arabia ordered from lowest to highest concentration of hydrocarbons.

Saudi Sand Cities	**Abbreviated Saudi Name**	**Extracted Aliphatic Hydrocarbon Range**	**Lowest Concentration mg/kg** ± SD	**Highest Concentration mg/kg** ± SD
Khobar	KO	C_{21} to C_{30}	1.9 ± 0.3	53.8 ± 11.4
Al-Hassy	HS	C_{21} to C_{29}	3.1 ± 0.56	123.6 ± 3.3
Jeddah	J	C_{21} to C_{31}	4.3 ± 0.8	131.7 ± 13.5
Riyadh	R	C_{20} to C_{31}	6.2 ± 1.4	278.4 ± 28
Skaka	SK	C_{21} to C_{30}	17.1 ± 2.5	410.6 ± 8.4
Unaizah	AN	C_{21} to C_{31}	23.2 ± 1.7	462.7 ± 47.8
Dammam	D	C_{21} to C_{30}	39.6 ± 2.1	781 ± 37
Tabuk	T	C_{21} to C_{31}	51.1 ± 3.2	1215.1 ± 61.1
Buraidah	B	C_{21} to C_{31}	91.4 ± 6.6	2316.6 ± 56.9
Hufuf	H	C_{21} to C_{31}	137.9 ± 10	3142.4 ± 57
Khafji	KF	C_{20} to C_{31}	38.2 ± 10.3	4176.4 ± 98.3
Sulayyil	S	C_{21} to C_{31}	205.1 ± 32	4062.8 ± 100.4
Najran	N	C_{21} to C_{31}	131 ± 28	5740 ± 141
Karj	K	C_{20} to C_{31}	40 ± 13.6	6978.1 ± 135

From the above figures (**Figure 25-28**), it was observed that C_{24}, C_{25}, and then C_{26} are the highest concentrations of aliphatic hydrocarbons in all sand samples respectively (**Figure 29**). The highest concentration in all samples was found in sample Karj with C_{24} = 6978.16 mg/kg, while the lowest concentration in C_{24} from all samples was found in sample Khobar at 53.87 mg/kg. The following figure (**Figure 29**) shows the variation in hydrocarbons for each sand sample. Showing the highest sample hydrocarbon quantification in sample Karj followed by Najran, Khafji then sample Sulayyil and the lowest sample is Khobar. This quantification of hydrocarbons was obtained by DCM 24 hours mechanical shaking.

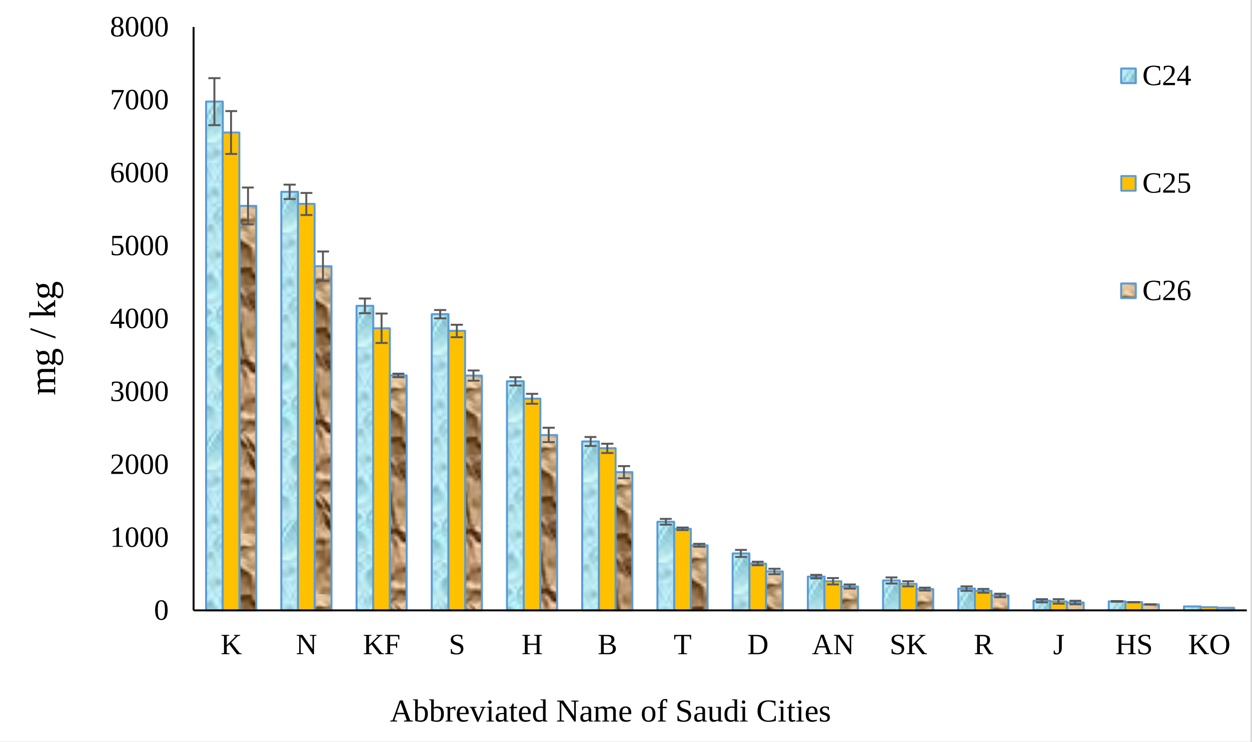


Figure 29 Expanded distribution of aliphatic hydrocarbon from C_{24} to C_{26} in the 14 Saudi sand samples.

The sand samples from the Saudi desert have a considerable amount of aliphatic hydrocarbons. These quantities of hydrocarbons in sand samples might constitute a hazard in the Saudi environment. Thus, some crucial factors play a role in the source of the hydrocarbons presence. One of them is that, Saudi Arabia is a country rich of hydrocarbons and has almost one third of the oil reserves for the entire world. Therefore, this reason might play a role in the presence of hydrocarbons in sand samples. In addition to that, some sampling sites are near oil refineries and that might contribute to the presence of hydrocarbons; it might come from some oil discharge or oil leakage from oil pipes. Therefore, this could be a contamination process and has to be considered. Scientifically, this contamination (hydrocarbons presence) might have severe effects in the environment and human health. The presence of the amount of hydrocarbons in sand might go through the sand down to the groundwater, especially during raining seasons. This would reflect negatively on the environment, plants, animals, and human health.

The weather in Saudi Arabia is an important factor; the temperature in summer is over 50 ^{o}C. This might contribute to the absence of hydrocarbons below C_{20} in all sand samples from Saudi Arabia, because the low molecular weight of hydrocarbons are evaporated faster than the high molecular weight ones. Therefore, there should be a solution regarding getting out the high molecular weight hydrocarbons from sand sites in order to remediate the sand. In this project, it is observed that high molecular weight hydrocarbons stick in sand samples for longer and more than low molecular weight hydrocarbons. The heat of sunlight in summer would evaporate the hydrocarbons below C_{20} and not evaporate the hydrocarbons above C_{20}. Therefore, this would explain the presence of C_{20} to C_{31} and the absence of C_{20} and below.

The government of Saudi Arabia should pay attention to the contamination of sand sites. This would affect and expose the environment to a hazard. Therefore, this project is aiming to get an applicable method for hydrocarbon remediation. This method would be applicable for small spillage of hydrocarbons and would help the society to get rid of the contamination.

2.3.9 Comparison between Water Extractable Hydrocarbons and Extractable Hydrocarbons using DCM (24 h Shaking).

Figure 30 shows the concentration of hydrocarbons extracted from sand using either DCM or water. Sample Dammam yielded hydrocarbons from C_{21} to C_{30} using DCM and the highest concentrations were found for C_{24}, C_{25}, and then C_{26} at 781 mg/kg, 642 mg/kg, and 534 mg/kg, respectively. The lowest concentration was found in C_{21} with 39.65 mg/kg. However, sample Dammam, using water extraction, extracted less hydrocarbons from C_{21} to C_{31}; the highest concentrations were found in C_{25}, C_{24}, and then C_{26} at 354 mg/kg, 335 mg/kg, and 322 mg/kg respectively. The lowest concentration was found in C_{21} at 29.20 mg/kg. In addition, sample Buraidah, using DCM extraction, yielded hydrocarbons from C_{21} to C_{31}; the highest concentrations were found in C_{24}, C_{25}, and C_{26} at 2316.61 mg/kg, 2222.75 mg/kg, and 1895.72 mg/kg respectively. The lowest concentration was found in C_{21} at 91.44 mg/kg. However, using water extracted fewer hydrocarbons from C_{21} to C_{31}. The highest concentrations were found in C_{25}, C_{24}, and C_{26} as 249.88 mg/kg, 240.04 mg/kg, and 293.95 mg/kg respectively, whereas the lowest concentration was found in C_{31} at 9.75 mg/kg followed by C_{21} as 14.55 mg/kg.

In addition, sample Sulayyil extracted hydrocarbons ranging from C_{21} to C_{31}, using DCM. The highest concentrations were found in C_{24} and C_{25} to be 4062.80 mg/kg and 3831.94 mg/kg respectively. The lowest concentration was found in C_{21} with 205.06 mg/kg. However, using the water extraction method, the hydrocarbons extracted ranged from C_{22} to C_{35}. The highest concentrations were found in C_{29} and C_{28} at 947mg/kg and 840 mg/kg, respectively; while the lowest concentration was found in C_{35} at 22.35 mg/kg. Regarding sample Hufuf, using the DCM method, extracted hydrocarbons ranged from C_{21} to C_{31}. The highest concentrations were found in C_{24} then C_{25} at 3142.43 mg/kg and 2902.47 mg/kg respectively. The lowest concentration was found in C_{21} with 137.93 mg/kg. However, using the water extraction method, the hydrocarbons extracted ranged from C_{23} to C_{36}. The highest concentrations were found in C_{29} then C_{30} at 1213 mg/kg and 1153.34 mg/kg; while the lowest concentration was found in C_{36} at 3.74 mg/kg. Using DCM for sample Riyadh, hydrocarbons extracted ranged from C_{20} to C_{31}. The highest concentrations were found in C_{24} followed by C_{25} at 278.45 mg/kg and 254.76 mg/kg, respectively. The lowest concentration was found in C_{31} at 6.23 mg/kg. However, using the water extraction method, the hydrocarbons extracted ranged from C_{23} to C_{34}. The highest concentrations were found in C_{29} followed by C_{30} at 114.34 mg/kg and 101.62 mg/kg, respectively. The lowest concentration was found in C_{23} at 8.03 mg/kg.

From the graphs in **Figure 30,** samples Dammam and Buraidah had almost the same pattern and same shape when using both methods. The highest concentration found in both samples was in C_{24} and then C_{25} using DCM; and C_{25} was the highest in both samples using the water extraction method. The lowest concentration was found in C_{21} for both samples. Regardless of the quantity of hydrocarbons in sand and its distribution, they have the same pattern. However, sample Sulayyil, Hufuf, and Dammam has a different pattern and there are differences in the total hydrocarbon concentration. The highest concentrations in all three samples using the DCM method were found in C_{24} followed by C_{25}, while the lowest concentrations were found in C_{35}, C_{36}, and then C_{23}, respectively. However, for the same samples, Sulayyil, Hufuf and Dammam, using DCM method the highest concentration was found in C_{29} for all the three samples. It seems there is a difference in the pattern of both methods. This might be because of the sand itself and the different locations. In addition the type of contaminants might explain the difference in extracted hydrocarbons shape and quantity.

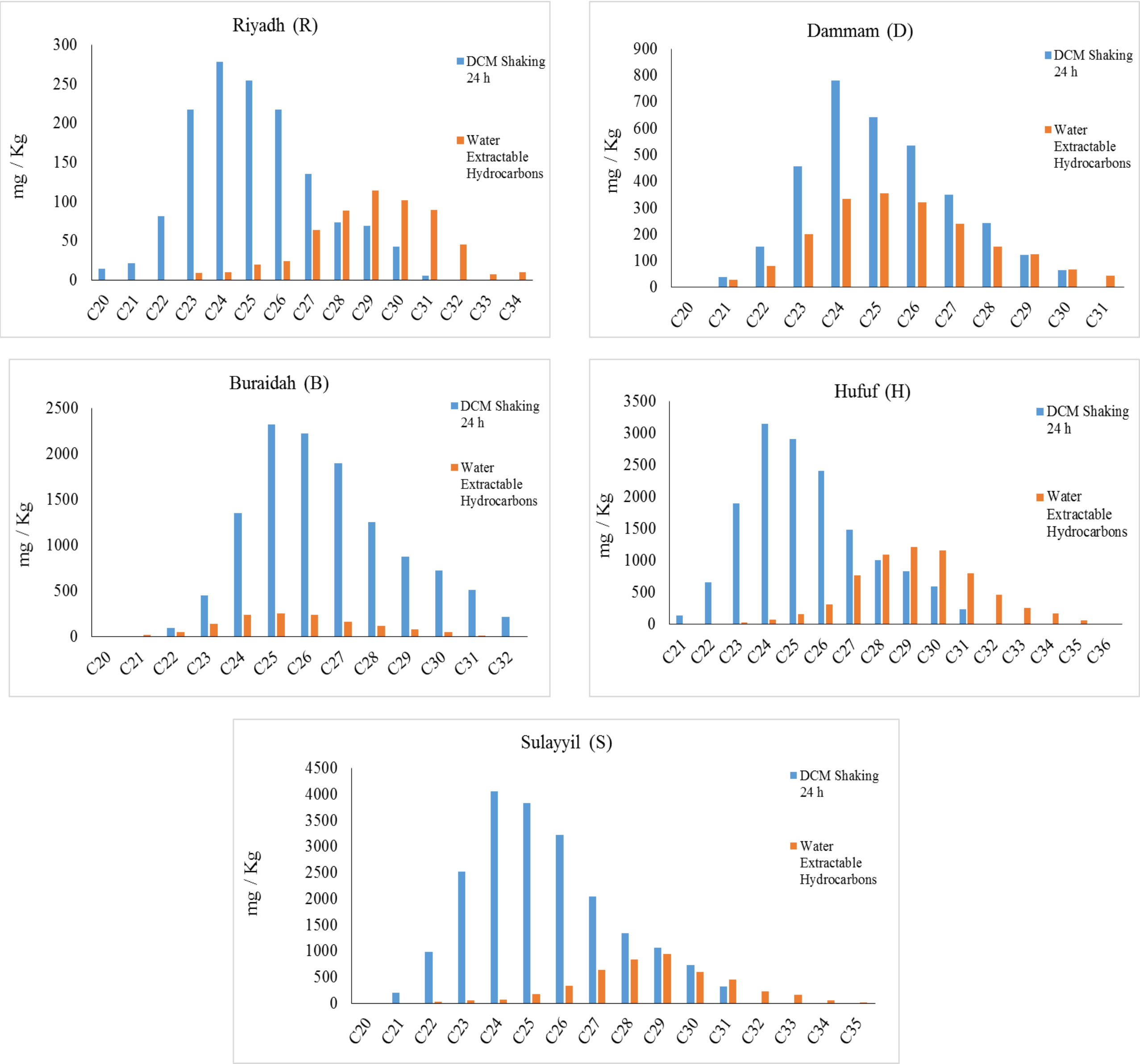


Figure 30 Comparison between DCM (Blue) and water extractable hydrocarbons (Orange) methods Dammam, Buraidah, Hufuf, Riyadh and Sulayyil; ordered from lowest to highest concentration of hydrocarbons.

It can be deduced that water extractable hydrocarbons could be detrimental to the environment. That is because during rainy seasons, water can miscible some hydrocarbons and take them down to the groundwater. In Saudi Arabia, rain season is limited and there is some shortage of rain. However, by analysing the sand samples, it has been found that some hydrocarbons could be extracted from sand using deionized water. That is an indicator that the groundwater may become contaminated by some types of hydrocarbons. According to this research, the method worked only for few samples, which might be because of the different type of sand samples, different type of contaminants, and different locations. Therefore, these reasons might be factors for not extracting hydrocarbons from some samples when using deionized water method.

2.4 Discussion.

In this section, the obtained results will be discussed in more details. In addition, results need to be compared to previous studies by other researchers.

2.4.3 Physio- Chemical Properties.

There was a variation in pH for Saudi sands as they ranged from 8.06 to 9.64; Saudi sands are alkaline in nature. The Saudi sand samples were compared to previous studies and there were high similarities in nature between the reported and previous studies (Khan, Hussein *et al.*, Hashem, 1993; Yasir, Azhar *et al.*, 2015 and Al-Oud, Nadeem *et al.*, 2011). However, there was a slight difference in some sample pH values compared with the literature. This reason could refer to different sampling times, amount of rainfall as at the time of sampling, type of area, and depth of sand collected. Previous work by Khan, Hussein *et al.* also found this alkaline nature. Sand from Abha city, Saudi Arabia was found to range between 6.75 to 8.78; only one sample was below pH 7. Hashem (1993) reported a similar pH range for sand from 10 different cities from Saudi Arabia (7.05 to 7.55). They found that the pH of sands samples from Riyadh and Dammam were 7.3 and 7.2, respectively. This was, however, slightly lower than was found in the sand samples used in the present study, but was within the same range. The pH of the sands used in this study was also in line with work by Yasir, Azhar *et al.* (2015), who studied sands from the southwestern highlands of Saudi Arabia. They reported a similar range of pH (7.7 to 8.3) for sands from 10 different places. These results further support that sands from this region (Saudi Arabia) are mostly alkaline in nature. It was reported that the pH of sand from Riyadh (Saudi Arabia) was 8.7, which is similar to the present study in the same city (Al-Oud, Nadeem *et al.*, 2011). The relatively high pH of the sand in this study may be due to different sampling times, amount of rainfall at the time of sampling, type of area, and depth of sand collected, because these factors have a significance in pH ranges.

In this project, sand moisture were slightly low compared to values reported in Khan, Hussein *et al.*, (2015), and they ranged from 0.04% up to 0.68%. However, Khan, Hussein *et al.*, (2015), reported a study that sand moistures ranged from 0.52% up to 1.2%. The difference in moisture content might be because of the amount of rain in Saudi cities and time of samples collections. As there is a shortage in rain in Saudi, the time of collection could play a significant role in the moisture content. Different cities are different in terms of amount of rainfall; Abha city has rain in winter time whereas other cities such as Riyadh and Dammam have less rainfall. Data on the moisture content in Saudi Arabian sands are limited but studies by Berlin *et al.* (1986), who examined the moisture content of sands collected from the Al Labbah region in northern Saudi Arabia and reported a range of 0.15 - 0.24% at 20 - 30 cm depth and 0.18 - 0.58% at 50 cm depth. The surface sand moisture content was reported to range from 0.054% - 0.077%. A study conducted by the United States Army (Ehlen 1993), also reported similar values, ranging from 0.38 – 0.58%, while samples collected during the rainy season were found to contain a slightly higher moisture content (1.8% - 3.7%) (Berlin, Tarabzouni *et al.* 1986). The moisture content in this project ranged from 0.04% to 0.68% and this is similar to the reported data. The moisture content in this study was slightly lower than was reported in previous studies in 2015 (Khan, Hussein *et al.*) for sand samples from Saudi, which ranged from 1.2% to 0.52%. The relatively lower moisture content reported in this study might likely be because of the variation for rainfall of different cities locations when samples were taken. In Abha city, rain is common, especially during the winter season, whereas the other cities have less chance of rain during the year.

Data on the moisture content of Saudi Arabian sands are limited, but studies by Berlin *et al.*, (1986), who examined the moisture content of sands collected from the Al Labbah region, in northern Saudi Arabia, reported a range of 0.15 - 0.24% at a depth of 20 - 30 cm and 0.18 - 0.58% at a depth of 50 cm. The surface sand moisture content was reported to range from 0.054% - 0.077%. A study conducted by the United States Army (Ehlen, 1993), also reported similar values, ranging from 0.38 – 0.58%, while samples collected during the rainy season were found to contain a slightly higher moisture content (1.8% - 3.7%) (Berlin, Tarabzouni *et al.*, 1986). The moisture content in this project ranged from 0.04% to 0.68% and is similar to the reported data

Saudi sands samples had a low organic matter contents, they ranged from 0.22% to 0.91%. The sand samples were collected from desert environments, the absence of plant or animal life is reflected in the low organic matter content. Yasir, Azhar *et al.* (2015) reported that the organic matter content for Saudi sands ranged from 0.21% to 1.75% in the southwestern highlands. Hashem (1993) conducted a study and found that organic matter content for Saudi sands ranged from 0.09% to 1.03%. Al-Oud, Nadeem *et al.* (2011) found that the organic matter content in Riyadh was 0.43%, which is similar to the present study for Riyadh, 0.39%. The results reported here support the relatively low organic matter content of sands from this region. Yasir, Azhar *et al.* (2015) reported that the organic matter content for sand samples from Saudi Arabia ranged from 0.21% to 1.75% in the southwestern highlands. Hashem (1993) also reported that the organic matter content for sand from 10 different cities in Saudi Arabia ranged from 0.09% to 1.03%. These results were similar to what is reported in the present study, most especially for Riyadh and Dammam. In the reported study the organic matter content in Riyadh was 0.05% and in Dammam was 0.63% whereas, in this present study, the organic matter content was 0.69% and 0.39% respectively. Al-Oud, Nadeem *et al.* (2011) stated that the organic matter content in Saudi Arabia (Riyadh) was 0.43%, which is similar to the present study for Riyadh, which was 0.39%.

2.4.4 Elemental Analysis (Mineral Content).

According to the conducted study, it has been found that ashing digestion was the best method to carry out elemental analysis in sand samples. In addition, the reason for the suggestion is that the ashing method extracts almost all elements, compared to aqua regia and nitric acid methods. However, there are still relative similarities between digestion methods in most samples. Therefore, these similarities would confirm that the three digestion methods could be applied for elemental analysis.

It could be because drying ashing using a Muffle oven at a high temperature (550 ^{o}C) for five hours then using nitric acid digestion gives a chance for extracting most or all elements from sand samples. However, the aqua regia and nitric acid methods has some similarities in some elements. Aqua regia could be stronger than just HNO_3 because it is a mixture of both nitric acid and chloride acid. From the Ca graph, it appears nitric acid is not an effective method to extract Ca for all samples while the other two methods extracted more and are similar. It was observed that the ashing method extracted the highest concentration for most elements compared to the other two methods. Elemental analysis for samples is stated (**See Appendix 2**). Therefore, this project suggest that using the ashing method for elemental analysis, as it appears better than the other two methods. In addition, the reason for the suggestion is that ashing method extracts almost all elements comparing to aqua regia and nitric acid methods. However, there is still relative similarity between digestion methods in most samples. Therefore, this similarity would confirm that the three digestion methods could be applied in elemental analysis method.

Kadi (2009), reported the total concentration of some elements such as K, Ni, and Zn from Saudi Arabia (Jeddah city) in 6 different sites using ICP-OES and ICP-MS was varied. Total concentrations ranged from 9227.2 mg/kg to 11439.8 mg/kg for K; from 9.13 mg/kg to 98.23 mg/kg for Ni; and from 56.59 mg/kg to 456.93 mg/kg for Zn. However, in the present study, the average concentration of K ranged from 55 mg/kg to 1386 mg/kg; Ni ranged from 0.37 mg/kg to 10 mg/kg; and Zn ranged from 2 mg/kg to 13 mg/kg. In the present study, lower concentrations of these elements were noticed when compared to previous work. For his analysis, Kadi collected samples from the roadside of heavily used highways in Jeddah. This illustrated the contamination of sand by Ni and Zn next to heavy traffic. In addition, the sand samples were prepared using a mixture of HF, HNO_3 and HCl, which could be the cause for the higher K content.

Al-Oud, Nadeem *et al.* (2011) stated that elemental analysis for sand samples from Saudi Arabia (Riyadh) was performed using ICP-AES. The reported study stated that the total concentration of Cu ranged from 0.4 mg/kg to 9.3 mg/kg whereas, the total concentration of Cu in the present study ranged from 0.6 mg/kg to 2.2 mg/kg. Moreover, the reported study stated that the total concentration of Fe ranged from 159 mg/kg to 6479 mg/kg, whereas the present study reported that the total concentration of Fe ranged from 134 mg/kg to 6839 mg/kg. In addition, the reported study stated that the total concentration of Zn ranged from 0.1 mg/kg to 42.6 mg/kg whereas, the present study stated that the total concentration of Zn ranged from 2 mg/kg to 13 mg/kg. The reported study stated that the total concentration of Al ranged from 277 mg/kg to 12060 mg/kg whereas, the total concentration of the Al in the present study ranged from 1065 mg/kg to 4575 mg/kg. Consequently, the four elements in both studies were higher in the reported study than in this present study. For his analysis, Al-Oud collected samples near a concrete factory. This might be a reason for the high concentrations in his samples when comparing to the current results. This illustrated that Zn, Cu, Fe, and Al in sand samples collected near a concrete factory are higher than sand samples collected from desert environments which are removed from human activities. Therefore the samples near to the factory might be contaminated and affected by the factory and the processes of producing concrete.

A technical report written by the US Army (Ehlen, 1993) on the physical properties of fifty-nine sand samples, taken from 0 – 15 cm depth, from the Empty Quarter in the south, eastern Saudi Arabia and from the Neutral Zone between Saudi Arabia and Iraq. The data from technical report also showed that over 40% of the samples analysed were classified as sand, 14.3% as fine sands, 31.4% as coarse sands, 5.6% as loamy sands, 2.8% as loamy coarse sand, and 2.8% as sandy loam. Petrographic analysis of the fine-grained sand samples analysed by Ehlen, 1993, showed that sands in Saudi Arabia is generally composed of approximately 30% quartz, <1% feldspar, 54% carbonate rock, 15% gypsum rock, 1% sandstone rock, <1% chert, <1% plutonic rock fragments, <1% mica and <1% heavy minerals (magnetite). From the northeast Saudi Arabia, the sands were found to be buff-coloured sand and consists of mainly 79% quartz, 6% potassic feldspar (i.e microcline, perthite, orthoclase,), 1% twinned plagioclase, 6% carbonate rock, 3% plutonic rock fragments (microcline/plagioclase/muscovite), 5% volcanic rock fragments (rhyolite), <1% mica (muscovite, biotite) and <1% gypsum.

In general, the authors found that carbonate, quartz and gypsum were the major component of the sand of the Middle Eastern region, while quartz, carbonate and feldspars dominated the sand in the central region of Saudi Arabia. The sand in the west was reported to be predominantly composed of quartz, feldspars, hornblende and mica. The mineral content of sand was also stated to be largely influenced by the rocks from which they were derived from. The sand in the west were mainly affected by the igneous and metamorphic rocks of the Arabian shields, while sedimentary rocks had great influence on sands in the central and eastern regions (Ehlen, 1993).

Therefore, according to Ehlen (1993) and his results, the current sand samples might composed of gypsum, feldspars, and hornblende as a high percentage of sand composition. Quartz and feldspar can be described as primary minerals, which composed of Si and $CaAl_2Si_2O_8$, respectively. In this project, Ca was the highest concentration with an average of 7384.7 mg/kg, which indicates to gypsum. The Al had an average of 1963 mg/kg and Fe an average of 2590 mg/kg. Mg had an average of 987 mg/kg and K an average of 545 mg/kg. It is expected that there should be a high concentration of Si, as sand is predominantly made from quartz. However, Si (**Appendix 3**) had a low concentration, 23 mg/kg for all sand samples - which is low using the three digestion methods. The reason could be because HNO_3, aqua regia or ashing is not capable of extracting Si from sand. Hydrofluoric acid might have the ability to extract more Si than other methods. However, in this research hydrofluoric cannot be applied in elemental analysis.

2.4.5 Fractionation of Hydrocarbons into Aliphatic and Aromatic Fractions Using Chromatographic Column Separation Method.

A chromatographic column separation method to separate hydrocarbon mixtures into an aliphatic and an aromatic fraction was successful using activated silica gel. The solution was separated into aliphatic and aromatic moieties using organic solvents. Hexane was used for the aliphatic fraction (F_1). A mixture of DCM-Hexane was used to get the aromatic fraction (F_2). This is recognisable because of the even spacing for the long chain hydrocarbons, as shown for the alkane standard solution above. Chromatograms of aromatic moieties differs from chromatograms of aliphatic moieties in the disappearance of the equally spaced alkanes and the increase in area of the remaining peaks. Comparison with the PAH standard, only resulted in three identifications based on comparison with the standard and confirmation using the MS, which were naphthalene, 2-methylnaphthalene, and phenanthrene. This confirms that the separation of the aliphatic from the aromatic fraction was successful.

Therefore, having hexane check solution after fraction 1 and fraction 2, could illustrate the volumes obtained in the aliphatic and aromatic fractions were appropriate and a clear separation between aliphatic and aromatic compounds in TPHs.

Seepage velocity of hydrocarbons through sand depends most on the water content of the sand. Seepage velocity of fuel depends on the type of sand. For example, using gasoline on different types of sand (gravely, peat, and sandy till), the seepage velocity of gasoline in gravely sand approximately was over 40 times greater than in peat sand and approximately 70 times greater than in sandy till. Using diesel fuel, the seepage velocity in gravely sand was around 30 times greater than in peat sand and was approximately over 40 times greater than in sandy till. Consequently, the seepage velocity with gasoline was three to five times faster than with diesel fuel (Halmemies, Gröndahl *et al.*, 2003). Therefore, the sand moisture content has a significant effect on seepage velocity, however, this effects does not affect its retention.

2.4.6 Determination of Percentage of Aliphatic and Aromatics in Crude Oil and Diesel.

The recovery percentage of crude oil and diesel was calculated gravimetrically. Diesel recovered approximately 80% of aliphatic and aromatic fractions. While crude oil recovered approximately 30% of aliphatic and aromatic fractions. This would suggest that about 10 to 20% of diesel oil contains volatile hydrocarbons (under C_9), polar compounds retained on the column, and possibly asphaltenes (hexane insoluble constituents). In addition, this suggests that about 70% of crude oil contains volatile hydrocarbons (under C_{12}), polar compounds retained on column, and possibly asphaltenes (hexane insoluble constituents). Therefore, the determination of the percentage of aliphatic and aromatic in crude oil by gravimetric methods ranged from 28.13% to 30.44% of the original total amount of crude oil.

Figure 20 shows that reducing the fractions obtained via the chromatographic methods to dryness results in a loss of the lower boiling hydrocarbons. This was addressed in two ways: the obtained fraction was not reduced to dryness and a procedural standard was added. The procedural standard was chosen based on a similar boiling point to the hydrocarbons eluting between 3 to 13 min and occupying a position in the chromatogram where there was a relatively empty space. The A graph shows fewer peaks than B and C graphs. This can confirm that crude oil compounds are volatile and during the evaporation process some compounds evaporate and approximately 10% to 20% might stick in the column.

Therefore, the determination of the percentage of aliphatic and aromatic in diesel by gravimetric methods can be worked out to determine the percentage of hydrocarbons recovery. This method recovered from 80% to 90% of the original total amount of diesel fuel. While crude oil recovered around 30% of the original crude oil.

2.4.7 Aliphatic Compounds in the Saudi Sand Samples (Leaching Experiment).

In this project, hydrocarbons in sand samples from C_1 to C_{20} were not detected in the leaching samples. This might be because the compounds are volatile, and the sand samples came from Saudi desert, where the temperature there can reach up to 50 ^{o}C in summertime. The high temperatures might be an effective factor for removing the lower hydrocarbons from sand samples through evaporation. In addition to these factor, the variation of sand locations and time of collection might have an effect, especially, as samples are over 2000 km distance.

However, in this project, it is realised that, the aliphatic hydrocarbons in some sand samples from Saudi are high, especially these hydrocarbon peaks extracted by a water leaching experiment. Water is not expected to extract hydrocarbons, however, it did in some samples. This means that there is contamination in these area and needs to be treated. This contamination might because Saudi Arabia is a rich country of oil and most of the desert has a huge amount of oil fields. Therefore, this might be a reason for the contamination. In addition, there might be some oil discharges in some area from oil refinery, which close to some area. However, this contamination might have side effects on the environment and human health. Therefore, attention should be paid to this contamination from the Government and Environmental Health Authority.

2.4.8 Quantification of Extractable Aliphatic Compounds in the Sand Samples Using DCM 24h Shaking.

In 2011, the United Nations Environment Programme (UNEP) conducted a study in Ogoniland and reported that the intervention value of mineral oil is 5000 mg/kg as set by EGASPIN (Environmental Guidelines and Standard for the Petroleum Industry in Nigeria). Therefore, any sample with a TPH concentration above 5000 mg/kg is considered to be contaminated according to UNEP and needs to be treated. In addition, the study also reported the intervention values as set by EGASPIN of benzene, ethyl benzene, phenol, toluene and xylene to be 1 mg/kg, 50 mg/kg, 40 mg/kg, 130 mg/kg and 25 mg/kg, respectively (United Nations Environment Programme (UNEP), 2011).

This study applies the same benchmarks set by EGASPIN for the concentration of TPH in the samples investigated and samples K (34707.8 mg/kg), N (28008 mg/kg), KF (20995 mg/kg), S (20347 mg/kg), H (15285 mg/kg), B (11911 mg/kg) and T (5935 mg/kg) are thus deemed to be contaminated samples. While samples D, AN, SK, R, J, HS and KO are below the contamination limit of TPH.

However, in 2017 Meshari reported that, the TPH of Kuwaiti oil contaminated sand ranged from 300,000 mg/kg to 350,000 mg/kg. The reported concentration of Kuwait samples was too high and by comparing the present concentration of TPH to the reported results, the present results should be not contaminated, because total aliphatic hydrocarbons in Saudi samples ranged from 236 mg/kg to 34708 mg/kg. Moreover, there is more than a magnitude difference in the total concentration in both studies (Almutairi, 2017).

The Kuwaiti oil contaminated sand had a high percentage of TPH and that due to the Gulf war in 1990, which resulted in 31,170,000 gallons of oil into the Kuwaiti desert. However, this percent of oil in the contaminated sand still has a massive difference by comparing it to the Saudi sand samples. Therefore, based on the level of TPH in Kuwait, the background of TPH in Saudi desert could be normal limit of hydrocarbons in sand samples, which not make any danger to the environment and human health. In addition, the Gulf war was 24 years ago, which means that most of hydrocarbon compounds with low molecular weight are volatile and would have evaporated already, especially the Kuwaiti desert heat, which can approach 50 °C in summer time. The compounds with complex and high molecular weight would exist in the oil contaminated sand, while some of them might have reached the groundwater in Kuwait (Almutairi, 2017).

Based on the reported results, the total concentration of aliphatic hydrocarbons in sand samples ranged from 236 mg/kg in sample KO to 34708 mg/kg in sample K. The average concentration of total aliphatic hydrocarbons for all sand samples was 10528 mg/kg. In addition, sample KF, N (the second highest concentration) and S had concentrations of 20995 mg/kg, 28008 mg/kg and 20347 mg/kg, respectively. Therefore, sample K is the only sample that might be classed as contaminated compared to other samples. Therefore, this project suggested that the spiking level should be 0.5% to 3% of crude oil and diesel. The reason of going up to 3% is that all samples are below 21000 mg/kg apart from sample K, which is 34708 mg/kg. Therefore, the hydrocarbons level in sand samples might be the normal limit of hydrocarbons in sand apart from sample K.

2.4.9 Comparison between Water Extractable Hydrocarbons and Extractable Hydrocarbons using DCM (24 h Shaking).

In this project, it has been found that water did extract hydrocarbons from some Saudi sands. This explains that when contamination occurs in the environment, rain could take the hydrocarbons contamination from the top of sand into the ground. Therefore, contamination needs to be treated as it occurs. In addition, the organic solvents extraction method (DCM) did extract more than water in all Saudi sands. Water extracted less hydrocarbons than using DCM shaking for 24 h (**Figure 30**). DCM is a polar non-protic organic solvent. Deionised water is a very polar solvent. Hydrocarbons are mostly non-polar hence a higher solubility with DCM was expected. However, there were some sand samples from which it was possible to extract some hydrocarbons using deionized water.

3 Chapter 3: Assessment of Ecotoxicity of Hydrocarbon Containing Sand using Seed Germination.

3.1 Introduction.

Hydrocarbon contamination in soil can influence plants indirectly interfering in the plant rhizosphere. Hydrocarbon pollution can cause loss of organic matter content (OMC), soil structure deterioration, and mineral nutrient loss (K^+, Na^+, $SO_4{}^{2-}$, $PO_4{}^{3-}$, $NO_3{}^-$). This pollution would have an effect on the environment, plants and possibly human health (Baruah, Deka and Baruah, 2016). Whether hydrocarbon contamination in sand is detrimental to plant growth requires a means of assessment. Due to the near absence of organic matter in the Saudi sands, a simple assay to establish is seed germination. Seeds provide their own nutrients for the formation of the seed leaves and the initial roots. There was no comparative assay described in the scientific literature, as they used soil, however, using plants for phytoremediation has been investigated. This method depends on the use of living green plants at specific sites in order to clean up a contaminated areas (Liu *et al.,* 2012). It has been pointed out that vegetation could play a significant role in hydrocarbon and toxic organic chemicals remediation. This type of remediation has been considered as one of the best developed technologies for remediating the environment from contaminants and metals from soil. In phytoremediation technology, suitable plant selection is considered as one of the key factors in the success of the process. The interaction between plant roots and rhizosphere play a crucial role in degradation process of organic compounds in soil (Baruah, Deka and Baruah, 2016).

Hydrocarbons, such as diesel and crude oil, can seep down into the soil after spillage. The physical properties of hydrocarbons limit the downward travelling of hydrocarbons throughout the environment (Adam and Duncan, 2002). The organic surface soil has an ability to adsorb hydrocarbons under ordinary conditions. Therefore, the contaminants (hydrocarbons) will be held in the surface soil, where most plants tend to set their root system. This would make hydrocarbons good candidates for phytoremediation. The availability of microbial proliferation in the rhizosphere would help plants with organic contaminants degradation in soil. The rhizosphere microorganisms and host plant can influence the degradation processes of contaminants in soil. If plants can be grown on a contaminated environment, then microbial interactions in the rhizosphere would breakdown hydrocarbons in vegetated soil. Therefore, plants that grow well in a polluted area would be crucial in phytoremediation technology (Cunningham *et al.,* 1997).

The phytoremediation is defined as the use of green plants and the associated microbiota in order to remediate an environment from polluted sediment, water and sand (Cunningham *et al.*, 1996). Phytoremediation systems is not new for remediating contaminated soil; the process of cleaning contaminated environment by plants dates back hundreds of years. The use of plants in soil remediation has become an area of great scientific research since Cunningham published his update on the potential in 1996 (Cunningham *et al.*, 1996). The rhizosphere microorganisms and host plant can influence the degradation processes of contaminants in soil. If plants can be grown on a contaminated environment, then microbial interactions in the plant rhizosphere would breakdown hydrocarbons in vegetated soil. Therefore, plants that grow well in polluted areas would be crucial in phytoremediation technology (Cunningham *et al.*, 1997).

According to Gudin and Syratt, 1975, grasses can be used in soil remediation because grass has a fibrous root system with great surface region for colonisation of microbes. These roots systems are able to form a persistent, intense rhizosphere, which leads to exemplary conditions for phytoremediation.

Adam *et al.*, in 1999 conducted a study on twenty two species of plants in a contaminated soil. The plants were measured 14 days after planting at 20 ^{o}C. The study examined the ability of plants in order to germinate in diesel-contaminated soil. It was noticed that there was a delay in seeds emergence and decrease in germination rate. The study illustrates differences between plant types regard to their ability in order to germinate in polluted soil. Most of herbs, legumes and commercial crops showed they were not affected by 25 g/kg of the contamination. Whereas, one type of grass (rough meadow grass) was intolerant at 25 g/kg. It was found that at 50 g/kg of contamination, half the plants did not exceed 50% of seed germination compared to the control rate. However, cocksfoot and rough meadow grass did not germinate at 25 g/kg of contamination (Adam and Duncan, 2016).

Baruah. P. *et al*. in 2016, conducted a study on a type of herb, called *crotalaria pallida*. The aim of the study was to evaluate the potential of the plant in remediating soil from crude oil. *Crotalaria pallida* grow in India in soils contaminated with crude oil. A set of crude oil concentrations, from 1% up to 10%, was spiked into soil. The outcome found that by increasing the concentration of crude oil in soil, the uptake of hydrocarbons by plants was increased. This increase was up to 6% of crude oil, after this limit, the study reported that the uptake of hydrocarbons by plants was lower with increasing concentrations of crude oil, from 6% up to 10%. In addition, the study also reported that, at a concentration of 10% of crude oil contaminated soil, the plant could not survive. However, the study reported that by increasing the concentration of crude oil, the plant shoots and roots biomass reduced (Baruah, Deka and Baruah, 2016).

In 2012, a study in China conducted experiments using 14 ornamental plants in order to assess the phytoremediation potential in petroleum contaminated sand (samples depth of 25 cm), by examining the effect on the degradation potential of both TPHs and its composition. This research reported, that the TPHs and its composition can be effectively reduced in 10,000 mg/kg TPH contaminated sand by pot-culture experiment of the 14 ornamental plants. The pH of contaminated sand (7.66 pH), as well as the concentration of C, P, N, and P (45.77, 0.65, 0.73, and 0.002 g/kg, respectively) were measured. The average concentration of TPHs in contaminated sand was approximately 28,000 mg/kg, the TPHs composition of aliphatic hydrocarbon fraction, aromatic hydrocarbon, asphaletene and polar fraction was 40.76, 27.02, and 30.82% respectively (Liu *et al.,* 2012).

For example, the removal rates after 30 days were 37.2% for *Gaillardia aristata*, 46.7% for *Echinacea purpurea (L.) Moench*, 49.4% for *Festuca arundinacea Schreb*, 41% for *combinedF. arundinacea*, and 37.4 % for *Medicago sativa Linn*. Regarding the removal rates of both TPH composition was 39.4%, saturated hydrocarbon 38.4%, aromatic hydrocarbon 45.1%, asphaletene 42.9%, and polar compounds 37.5% (Liu *et al.,* 2012). Therefore, phytoremediation using different ornamental species is considered as an effective method of treatment of TPH degradation in petroleum contaminated sand.

Some specific plants have noted a noticeable reduction on the TPHs of contaminated sand. The removal rate ranged from approximately 19.4% to 49.2% in contaminated sand after remediation. Plants of *Echinacea purpurea (L.) Moench* and *Festuca arundinacea Schreb* had the largest percentage of the removal rate of TPHs, whereas, plants of *Centaurea cyanus L* and *Antirrhinum majus L* had the lowest percentage of the removal of TPHs. The removal rate of most of the other samples may reach more than 35% of the tested plants (Liu *et al.*, 2012).

Saudi Arabia lies perfectly in the hyper-arid and arid regions of the Arabian Peninsula (Almazroui *et al*., 2017). It is mostly arid country with, at least one–third of its land is desert of which less than 1% of the total area is suitable for cultivation only under irrigation. The area is characterised by irregular, heavy rainstorms which blow only a few millimetres of water per year and is usually restricted to some districts of the Saudi Arabia. In the inland regions, however, during the summer the heat is intense, reaching an average temperature of between 45°C - 54° C in places (Almazroui *et al.*, 2017). The heat is usually intense shortly after sunrise and lasts until sunset. In the spring and autumn, the heat is moderate, with temperatures average around 29°C.

As the background concentrations of hydrocarbons were found above some guidelines, this chapter investigates the ecotoxicological effects of the Saudi sands on the growth prolife (germination) of seeds from seven types of plants (radish, lettuce, coriander, wheat, ryegrass, cabbage red drumhead and cabbage greyhound). Plants were implanted in Saudi sands, control sand and contaminated control sand in both the UK and in KSA in order to evaluate the impact of the difference in climate on seed germination and root and shoot growth. In addition, the plant growth profile (shoot and root measurement) was studied in control sand, contaminated control sand and Saudi Arabian sands, for comparison.

3.2 Materials and Methods:

3.2.1 Materials

All chemicals were of reagent grade and have been used without further purification. Analytical grade hexane and dichloromethane (DCM) were purchased from Fisher scientific. All of the working solutions were prepared at different concentrations from the stock solution in hexane. Diesel oil (commercial petroleum) was purchased from petrol station in North Wales (Esso Tesco Chelsea Express). Crude Oil was donated from Dr Charlie Shand, James Hutton Institute, UK. Sodium sulfate anhydrous and silica gel were obtained from Fisher Scientific. Saudi Sand samples were collected from sixteen different cities in the Kingdom of Saudi Arabia (**Figure 10** (**page No 31**)). Horticultural sand was used as control sand (CS) and purchased from Treborth Garden Centre. Control sand used in each run of seeds germination in order to compare with contaminated horticultural sand and with Saudi sands in seed germination. Plant seeds **(Table 14)** were purchased from Suttons and Emors gate seeds. Wheat seeds were obtained from Bangor University farm.

Table 14 Seven different types of seeds plant.

Seeds Name	Latin Name	Source
Radish Seeds - French Breakfast 3	*Raphanus sativus*	Suttons*
Lettuce Seeds - Vailan - Winter Gem	*Lactuca sativa*	Suttons*
Herb Seed - Coriander	*Coriandrum sativum*	Suttons*
Wheat	*Triticum*	Bangor University Farm
Perennial Ryegrass (w)	*Lolium perenne*	Emors gate seeds
Cabbage Seeds - Red Drumhead	*Brassica oleracea capitata 'Red Drumhead*	Suttons*
Cabbage Seeds - Greyhound	*Brassica oleracea var. capitata*	Suttons*

*Seeds samples obtained from Suttons Seeds UK.

3.2.2 Sand Contamination with Crude Oil

Control sand (400g) was mixed with 0.5%, 1%, 2%, 5% and 10% of crude oil (þ=1/1.36 g/ml) to achieve different levels of contamination. To achieve the different percentages of crude oil in sand, 2 g, 4 g, 8 g, 20 g and 40 g of crude oil was added to the desired volume of a DCM-hexane (1:1) mixture in 1000 ml conical flasks. Crude oil was dissolved in the DCM-hexane mixture by mechanically shaking for 2 to 3 minutes. Next, the mixture was added to the sand (400g) and shook for a further 30 minutes. The contaminated sand was left at room temperature to allow the hexane to evaporate for 24 hours. The samples were then shaken again for 3 minutes and left uncovered for at least 24hours to assure complete removal of the hexane and DCM (Tang *et al.,* 2011) (Baruah, Deka and Baruah, 2016).

3.2.3 Seeds Germination

Contaminated and uncontaminated sand (10g) was added into petri dishes and 20 seeds of the desired plant were added on top of the sand along with deionized water (4 ml). All petri dishes were covered with lids and filter paper in order to block out light. All petri dishes were kept in a growth cabinet at 20 ^{o}C, and exposed to 16 h daylight and 8 h of darkness (Gmitrzuk and Dąbrowski, 2005). Seed germination was observed daily and recorded using photography. Water was added as needed. The seed germination percentage was calculated taking account of non-germinated seeds in the petri dish (Tang *et al.,* 2011*)* (Gmitrzuk and Dąbrowski, 2005). Once the leaves started to emerge, the cover (filter paper) was removed. Once both roots and shoots were growing, the plant was removed from the petri dish for further measurments. For the *dicotelydons*, once the seed leaves were fully expanded, from 7 days to 12 days depending on the type of seeds, the length of the roots and shoots were measured. For the *monocotelydons*, once the first blade was between 3 to 5 cm long, the root and blade length was measured.

3.2.4 Root and Shoot Examination

Roots and shoots were measured using RS Pro 150mm Digital Caliper 0.0005 in, 0.01 mm, Metric & Imperial. The root was measured from its end up to the seed. The shoot was measured from the end of the leaf down to the beginning of root. The measurement values appear as a digital number and were recorded in an observation sheet. An average of shoots and roots length was calculated with standard deviation and standard error using Microsoft Excel.

3.2.5 Soxhlet Extraction

In order to assess changes in the TPH composition of the sand during seed germination, the sand from replicated seed germinations at the same level of contamination was combined. Soxhlet extraction was performed using 20 g of each sand sample, which were placed into a thimble, inside the Soxhlet apparatus. DCM (150 ml) of was added into a round bottom flask and Soxhlet extraction was performed for 3 h. After the extraction, the solvent was collected and left to cool for 30 minutes. Then the solvent was removed using a rotary evaporator until dry. The dry extract was then dissolved in hexane (1 ml) containing an internal standard and transferred into a GC vial and sent for GC-MS analysis (Baruah, Deka and Baruah, 2016) (Okop and Ekpo, 2012) (Khan, Troquet and Vachelard, 2005).

3.2.6 DCM Extraction (24 h)

Sand samples (10 g) were placed into conical flasks (250 ml) and DCM (20 ml) was added. The conical flasks were sealed by parafilm and left shaking at 250 rpm for 24 h. The soluble fractions were collected in a round bottom flasks and solvent evaporated until dry. The residue was dissolved in hexane (1 ml) and the solutions transferred to GC vials and sent for GC-MS analysis (Schwab *et al.*, 1999).

3.3 Results

In this section all seed germination results will be discussed. The results varied depending on the type of seed, sand and contamination level. In addition, control sand was spiked with different concentrations of crude oil, ranging from 0.5% up to 10%.

3.3.1 Percentage Germination in Control and Contaminated Sands Conducted in the UK.

Twenty of each seed type were planted in control (**Figure 31 A**) and contaminated sand containing different concentrations of crude oil (0.5%, %, 2% 5% and 10% in **Figure 31 B, C, D, E, and F**) and the number of seeds that germinated was counted (**Figure 31**).

As can be seen from **Figure 31**, different seed varieties germinated to different extents in the control sand. **Figure 32** illustrates the percentage of seeds that germinated. Lettuce had the best germination in the control sand (92%) closely followed by Ryegrass (88%), Cabbage Greyhound (77%), Coriander (73%), Cabbage Red Drumhead (55%), Radish (55%) and Wheat (43%).

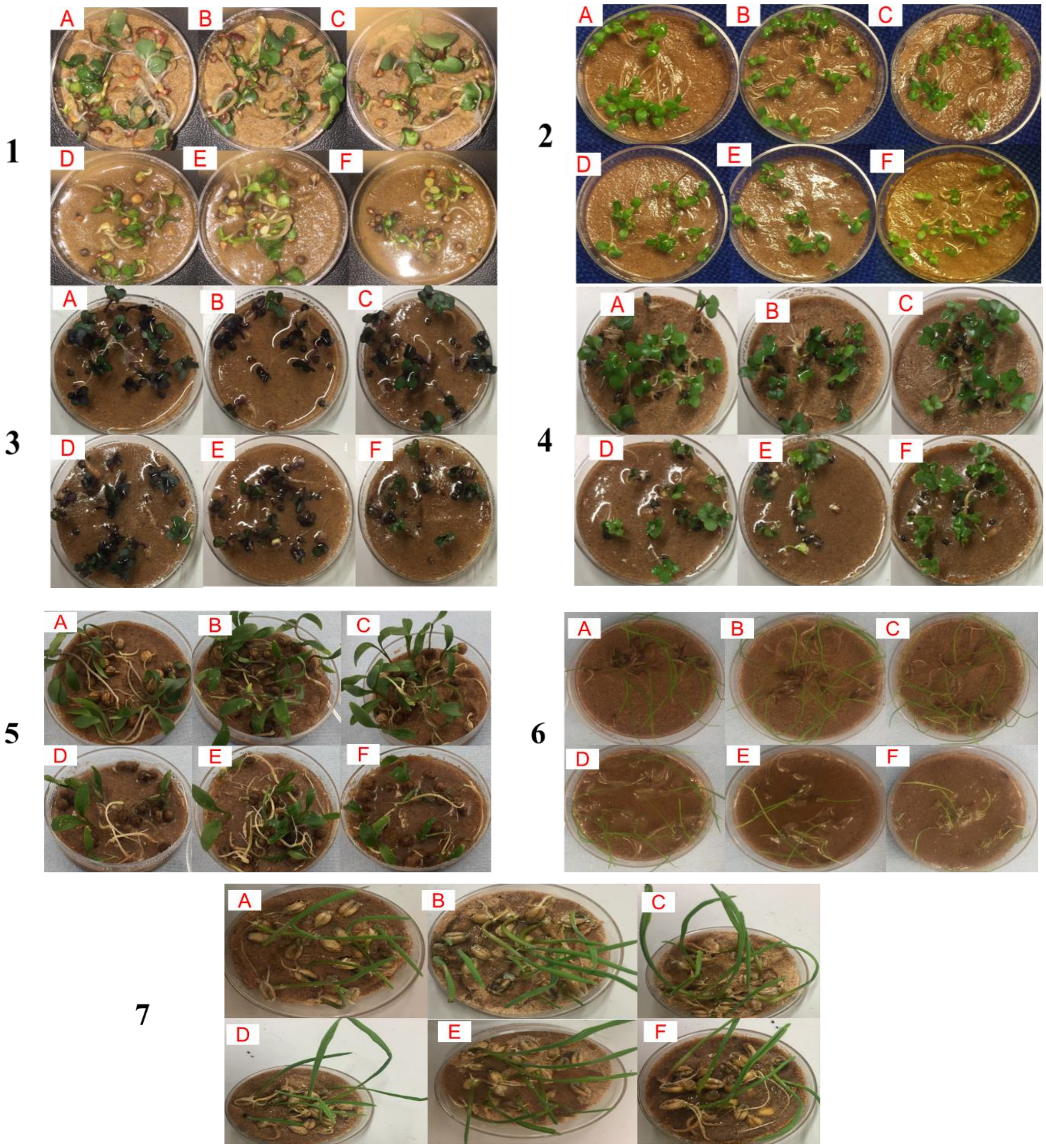


Figure 31 Seed germination of Radish (1), Lettuce (2), Cabbage Red Drumhead (3), Cabbage Greyhound (4), Coriander (5), Ryegrass (6) and Wheat (7) after being planted in sands containing different levels (0% [A], 0.5% [B], 1% [C], 2% [D], 5% [E], and 10% [F]) of crude oil contamination. (from top left to right).

At 0.5% and 1% of crude oil contamination, Lettuce had the highest percentage germination among plants (100%, **Figure 32**). Germination for Radish ranged from 30% (at 5% and 10% crude oil) to 50% (at 1% crude oil). Cabbage Red Drumhead germination ranged from +45% to +69% at contamination levels of 0.5% and 1% respectively. Cabbage Greyhound germination was 32% and 88% at crude oil concentrations of 5% and 10% respectively.

Coriander had 90% germination at a crude oil concentration of 0.5%, but germination decreased to 12% at a 5% level of contamination. Ryegrass had a 92% germination rate at a concentration of 1% crude oil, but only 48% at 10% crude oil contamination. Wheat showed poor germination in sand with only 48% of seeds germinating at a 0.5% crude oil and 28% germinating at a crude oil concentration of 2% (**Figure 32**).

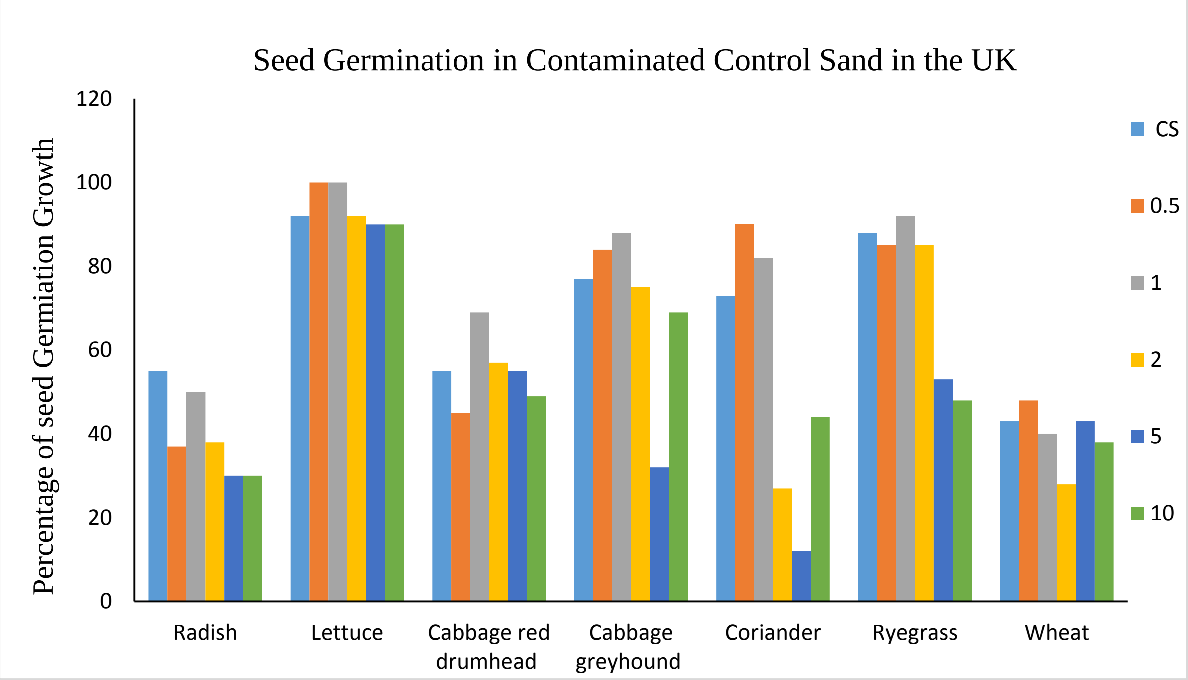


Figure 32 The percentage of seeds germinated in control and crude oil contaminated sand (0.5%, 1%, 2%, 5% and 10%). Germination was conducted in the UK.

3.3.2 Seedling Growth in Uncontaminated and Crude Oil Contaminated Sand in the UK.

All seedling growth experiments for uncontaminated and crude oil contaminated sand were conducted under the same conditions for consistency. Root and shoot length of the seven types of seedlings in contaminated sand were compared to root and shoot length in the control sand (**Figure 33** and **34).**

As seen from **Figure 33**, the root lengths of Wheat and Lettuce seedlings increased in all contaminated sand. Wheat root length increased by 111% and Lettuce root length increased by 58% at 2% crude oil contamination. To the contrary, Radish, Cabbage Red Drumhead and Cabbage Greyhound had a significant reduction in root length at all crude oil contamination levels. Radish root length decreased up to 88% at 10% contamination (**Figure 33**), while Cabbage Red Drumhead root length decreased up to 74% at 5% contamination (**Figure 33**). Cabbage greyhound had a similar reduction in root length to that of Cabbage Red Drumhead with up to 78% at 5% contamination. The root length of Coriander and Ryegrass was minimally affected at contamination levels of 0.5%, 1% and 2%, but decreased by 50% for Coriander at 5% contamination (**Figure 33**) and 27% for Ryegrass at 10% contamination (**Figure 33**).

Figure 33 and **34** show that the p value of all plants had been analused for roots and shoots of control sand and all level of contamination of control sand compared to each other. The result (**Figure 33**) for radish showed that there was no significant difference between roots at 0.5 % and 2 % ($p < 005$), also there was no significant difference between 2 % and 5 % as the p value < 0.05. In addition, there was no significant difference between shoots at 0.5 % and 2 % and 5% ($p < 005$). However, there was a significant diffeernce between shoots at 5 % compared to control sand and all levels of contamination apart from at 0.5%.

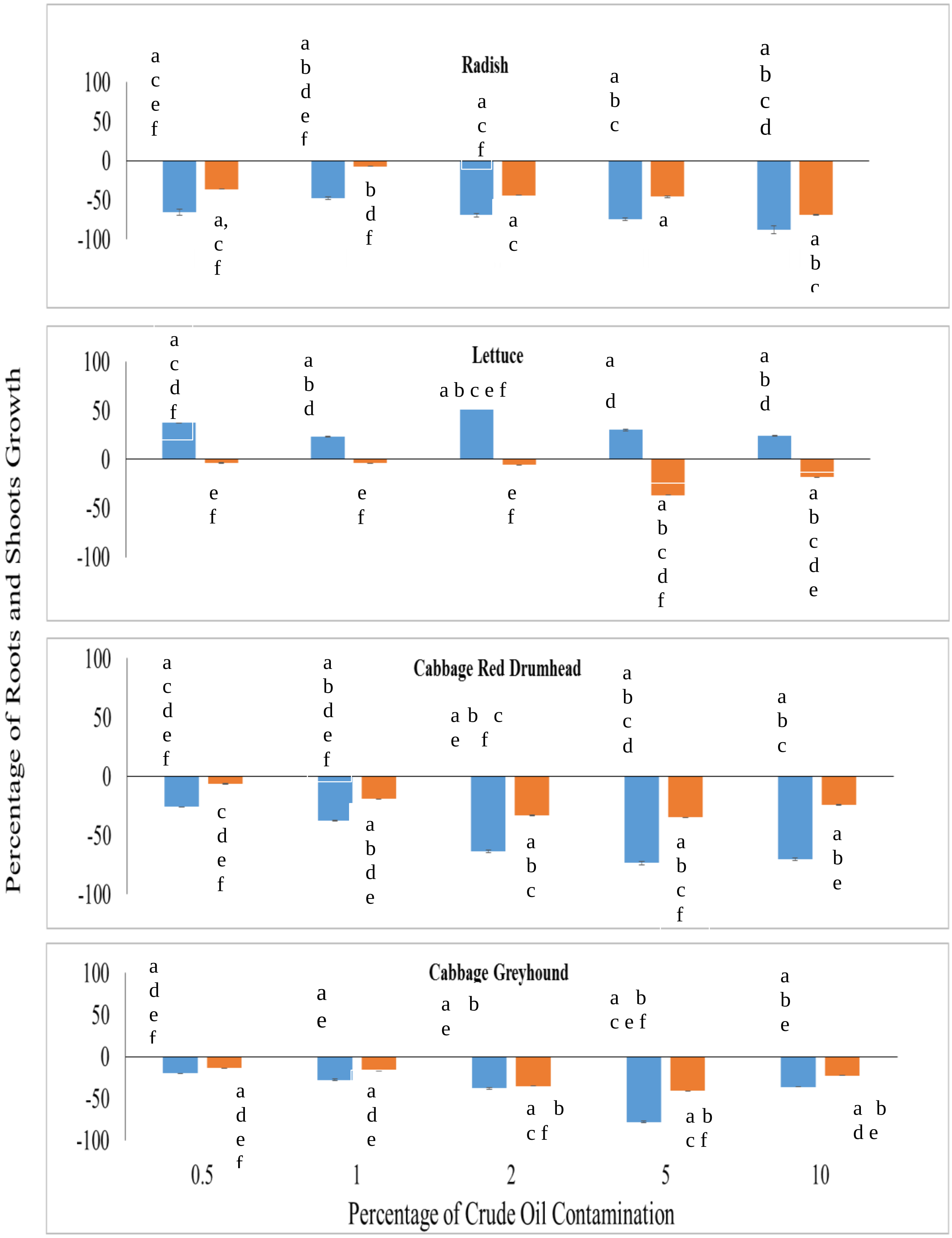


Figure 33 Root (blue bars) and shoot (orange bars) length of radish, lettuce, cabbage red drumhead and cabbage greyhound for seeds grown in different crude oil contaminated sands, expressed as a percentage of the root and shoot length for seedlings grown in control sand conducted in the UK. Error bars are 1 SEM (n=3). Lettering a, b, c, d, e and f refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with “a” when compared to the control sand, “b” when compared to 0.5 %, “c” when compared to 1 %, “d” when compared to 2%, “e” when compared to 5 % and “f” when compared to 10 % of crude oil contamination.

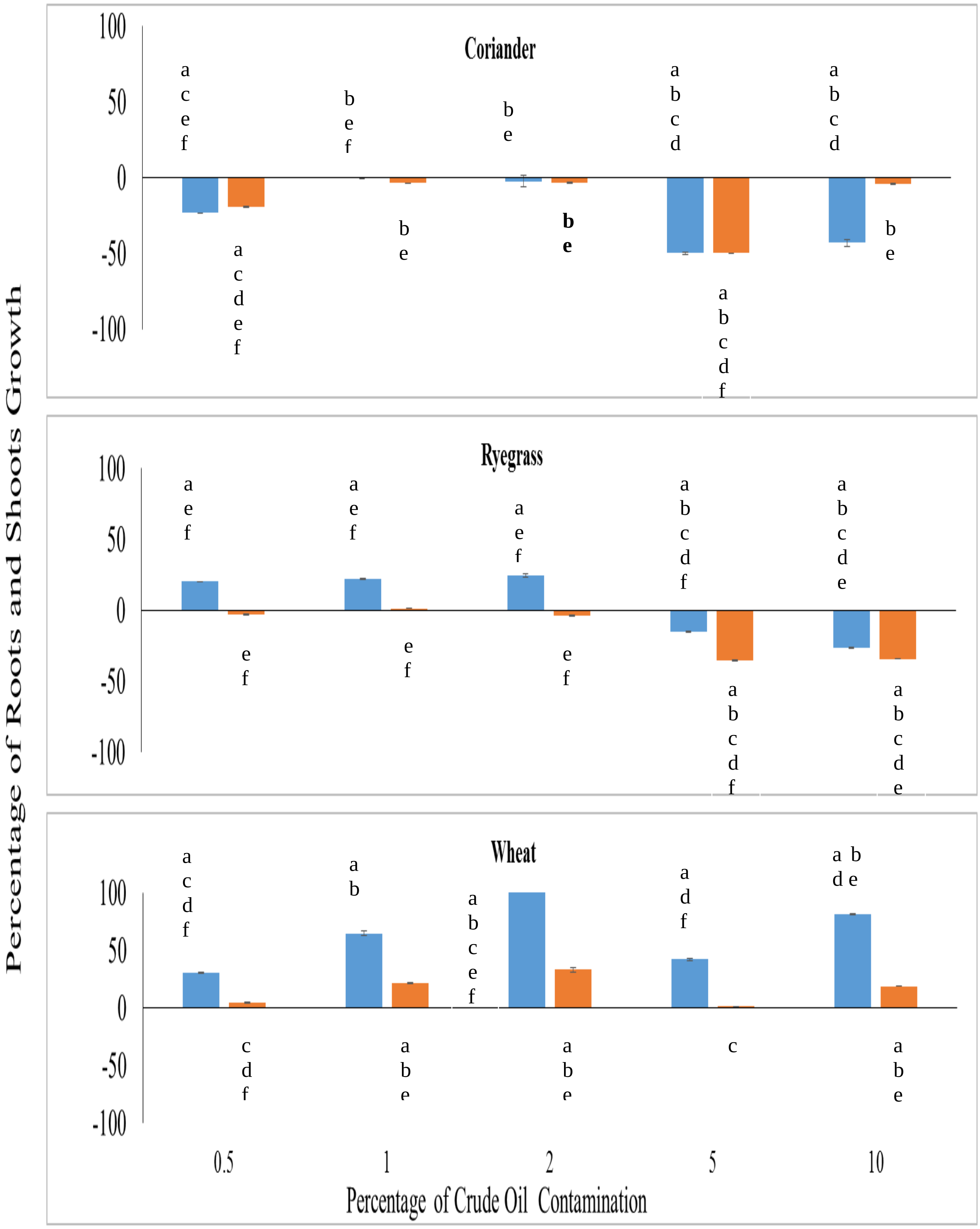


Figure 34 Root (blue bars) and shoot (orange bars) length of coriander, ryegrass and wheat for seeds grown in different crude oil contaminated sands, expressed as a percentage of the root and shoot length for seedlings grown in control sand conducted in the UK. Error bars are 1 SEM (n=3). Lettering a, b, c, d, e and f refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with “a” when compared to the control sand, “b” when compared to 0.5 %, “c” when compared to 1 %, “d” when compared to 2%, “e” when compared to 5 % and “f” when compared to 10 % of crude oil contamination.

As seen from **Figure 33**, shoot length for Wheat showed an increase at all levels of contamination. The most significant increase in Wheat shoot length of 10%, 33% and 5% was seen at 1%, 2% and 10% contamination levels respectively.

The effect of crude oil contamination on shoot length was similar for Lettuce, Coriander and Rye grass. Lettuce shoot length appeared mostly unaffected by crude oil contamination up to 2%, while 5% and 10% contamination levels caused a decrease of 37% and 18% respectively. Similar results were seen for Coriander, with a minimal effect on shoot length up to 2% crude oil contamination, but a decrease of up to 50% at 5% and 10% contamination levels. The same trend was seen for Ryegrass, with the only significant reduction (40%) seen at 5% and 10% contamination levels.

The shoot length for Radish, Cabbage Red Drumhead and Cabbage Greyhound decreased at all contamination levels, but most significantly at contamination levels at and above 2%. For Radish, the shoot length decreased by 8% at 1% of crude oil and 69% at 10% contamination. Cabbage Red Drumhead shoot length decreased by more than 25% at contamination levels above 2%. The shoot length for Cabbage Greyhound followed the same trend as for Cabbage Red Drumhead with a reduction in shoot length of 41% at 5% crude oil contamination.

Wheat and lettuce were found not to be susceptible at all contamination levels. Ryegrass up to 2% is not susceptible, whilst at contamination levels of 5 and 10% they are susceptible to crude oil. Wheat and lettuce had a positive response for roots at all contamination levels. Wheat is the only plant which had a positive response for shoot length at all contamination levels. Cabbage red drumhead and cabbage greyhound were found to be highly similar in terms of roots and shoots length at all contamination levels. Cabbage red drumhead and green were similar and very susceptible to crude oil contamination, as were radish and coriander. In addition, lettuce root growth was not susceptible to crude oil contamination and this could infer that the lettuce roots trying to find clean source of sand.

3.3.3 Seeds Germination in Saudi Sands Conducted in the UK.

The methodology used for control and crude oil sand in section 3.3.1, was next applied to each of the Saudi Sands. Into each of the 16 Saudi sands, twenty seeds were planted, and the number of seeds germinating was counted and expressed as a percentage out of 20. This was done for each of the seven seed types and all 112 experiments conducted in the UK (**Figure 35** and **36**).

As can be seen from **Figure 35** and **36**, Radish in sample Dammam had the lowest germination rate among all samples. Lettuce and Ryegrass generally had the highest germination rate compared to the other seeds. To the contrary, Wheat had overall the lowest germination rate with less than half of seeds germinating in 9 of the Saudi sands.

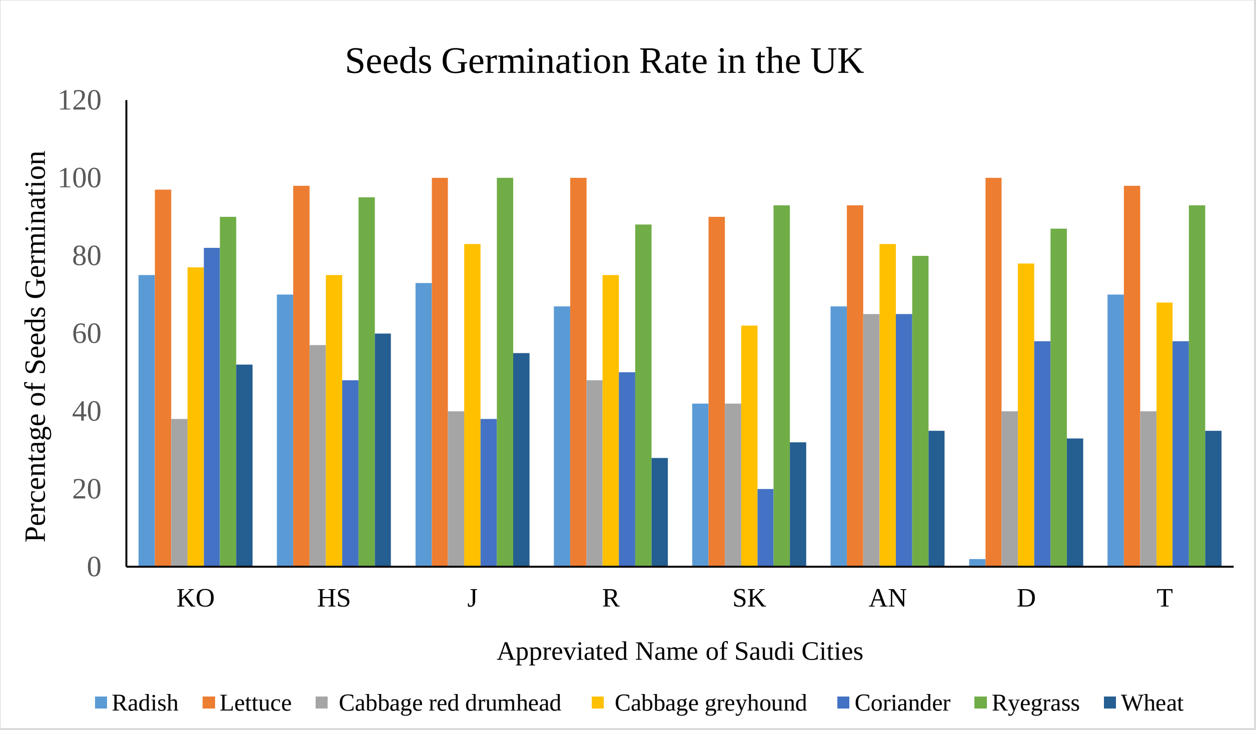


Figure 35 Seeds germination percentage in Saudi sands sampled from KO, HS, J, R, SK, AN, D and T conducted in the UK.

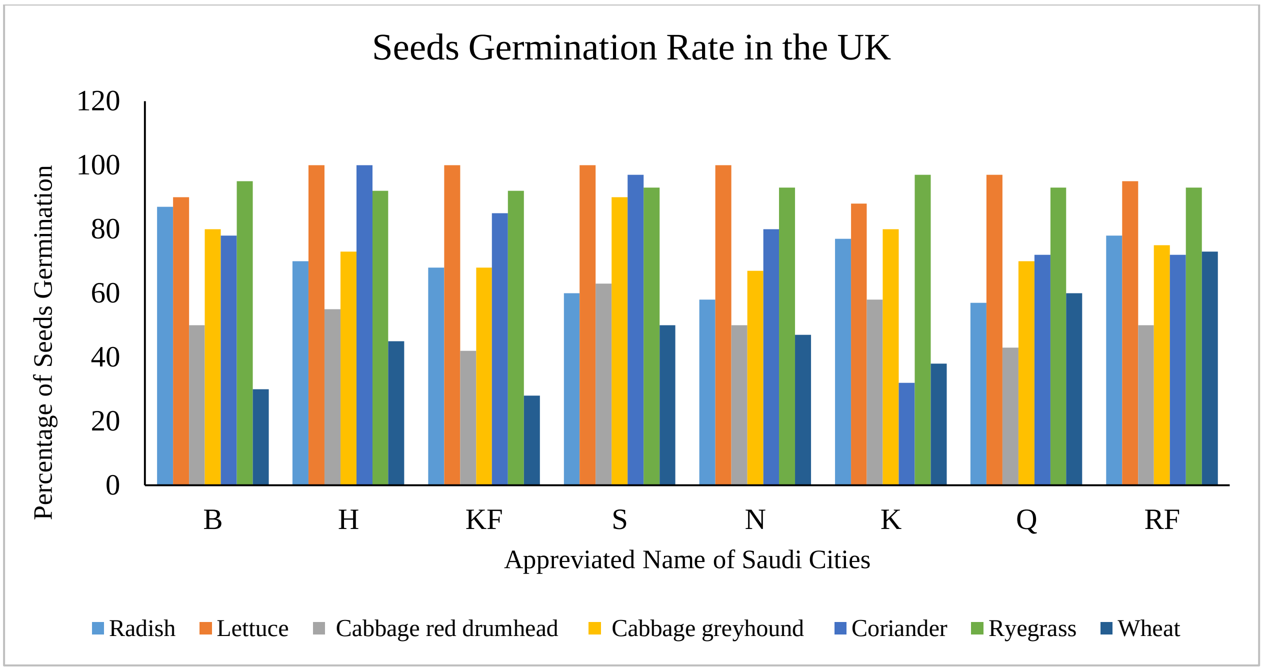


Figure 36 Seeds germination percentage in Saudi sands sampled from B, H, KF, S, N, K, Q, and RF conducted in the UK.

3.3.4 Seedling Growth for All Saudi Sand Samples Conducted in the UK.

Next, seedling root and shoot growth for each seed type grown in different Saudi sands was determined and compared to root and shoot length in the control sand (**Figures 37-43**).

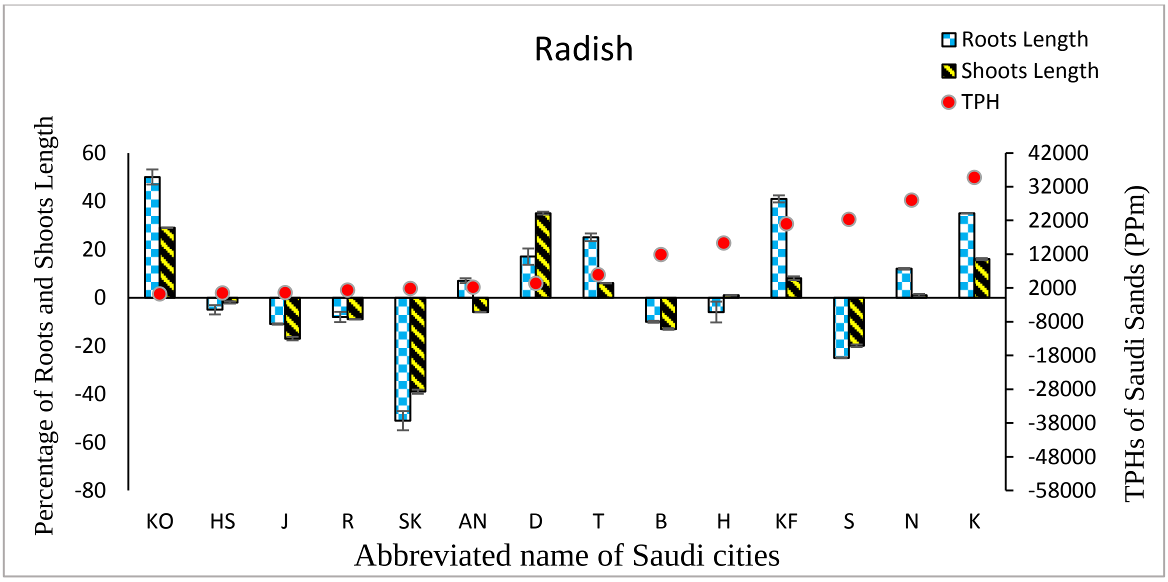


Figure 37 Root and shoot length of Radish seedlings grown in all Saudi sands compared to root and shoot length in the control sand. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

As seen from **Figure 37**, Radish grew in all Saudi sand samples, however, there was significant variation in root and shoot length of the seedlings when compared to the control sand (results not shown). The root length decreased in 7 of the Saudi samples and increased in the other 7, while the shoot length decreased in 6 of the sand samples and increased in the other 8 samples. The greatest decrease in root length (-51%) and shoot length (-39%) was seen for the Skaka (SK) sand sample, while the greatest increase in root length (+50%) was seen in the Khobar sample and greatest increased in shoot length (+35%) was seen in the Dammam sample.

The total concentration of hydrocarbons in part per million (ppm) for each Saudi sand was also plotted against root and shoot length for Radish (**Figure 37**). Contrary to what was expected, there was no linear correlation between Radish root and shoot length and the total hydrocarbon content of the Saudi sands. This suggested that Radish growth was affected by factors other than TPH in the Saudi sands.

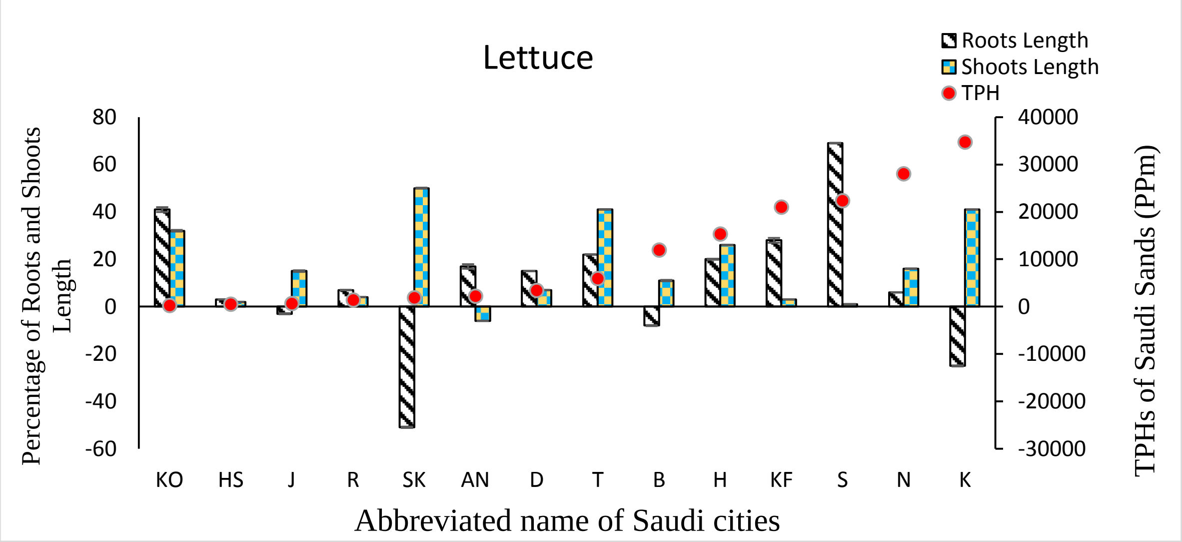


Figure 38 Root and shoot length of Lettuce seedlings grown in all Saudi sands compared to root and shoot length in the control sand. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Lettuce seedlings generally grew better in the Saudi sands compared to the control sand (**Figure 38**). The greatest increase in root length (+69%) was seen in the Sulayyil sample and the greatest increase in shoot length was seen in the Skaka sample. However, Lettuce root length significantly decreased (-51%) in the Skaka and Karaj sample, while the only significant decrease in shoot length was seen in the unaizah sample.

Again, there did not appear to be a linear correlation between the TPH content and the root and shoot length of Lettuce seedlings (**Figure 38**). Lettuce root length did however increase in the Sulayyil sample which had a TPH concentration of 20347 mg/kg corresponding to the 2% crude oil sample (**Figure 33**). Similarly, Lettuce shoot length remained virtually unchanged compared to the control sand in the Al-Hassy (HS), Jeddah (J), Riyadh (R), Unaizah (AN) and Dammam (D) Saudi samples (**Figure 38**) which had TPH concentrations corresponding to 0.5% and 1% crude oil (**Figure 33**).

Figure 39 shows the root and shoot length for Cabbage Red Drumhead grown in Saudi sand samples. The greatest decrease in root length (-30%) was seen in the Buraidah sample, whereas the greatest increase (+53%) in Cabbage Red Drumhead root length was seen in the Al-Hassy sample. Shoot length varied from - 9% in the Dammam sample up to + 45% in Al-Hassy sample. These results were significantly different from the results seen for the crude oil contaminated samples in which root and shoot length decreased at all contamination levels tested.

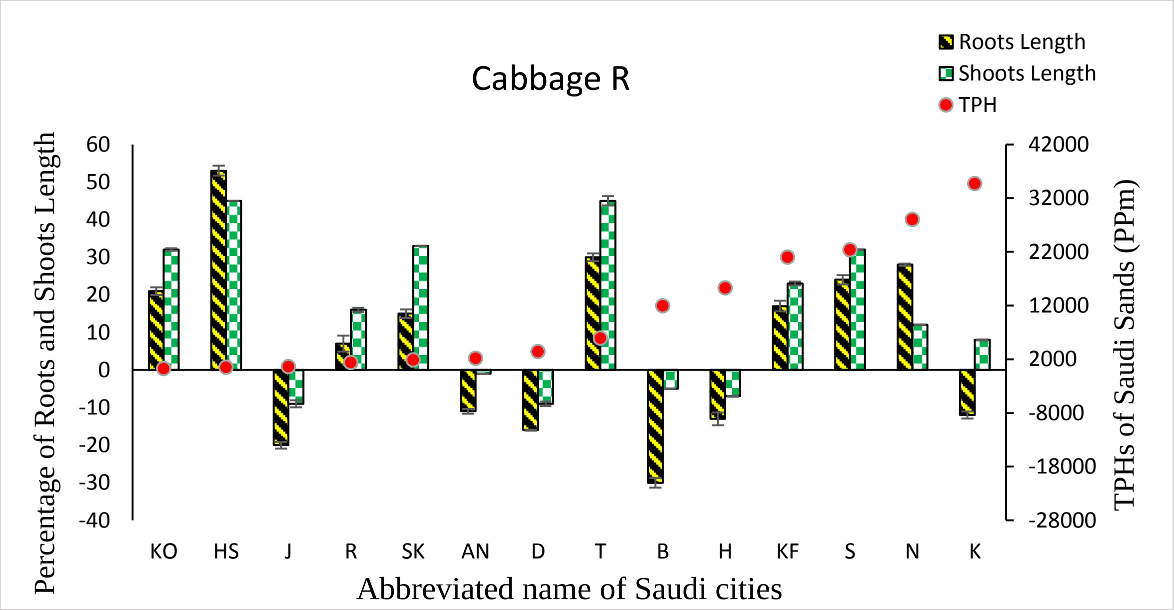


Figure 39 Root and shoot length of Cabbage Red Drumhead seedlings grown in all Saudi sands compared to root and shoot length in the control sand. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Similarly, for Radish and Lettuce, no linear correlation was seen when comparing root and shoot length to the TPH within the Saudi sands. Therefore, growth could just be an indication of the preference of seeds for one or more Saudi sands. It was also thought that seed size might play a role in the germination process.

Cabbage Greyhound is another example of the difference between seedling growth in Saudi sand samples (**Figure 40**) compared to crude oil contaminated samples **(Figure 33**). Increased root length was seen for Cabbage Greyhound in all Saudi sands except for “Jeddah”, “Riyadh” and “Najran”. The greatest root length increase of 57% was in sample Qatif and the greatest decrease of 12% was in sample Riyadh. This is in stark contrast to crude oil contaminated sands in which root length decreased at all levels of contamination. Shoot length for Cabbage Greyhound was also significantly increased in all Saudi sands except for Riyadh, Buraidah and Najran samples. The shoot length decreased by 13% in sample Najran, but increased by 83% in sample Khafji (**Figure 40**).

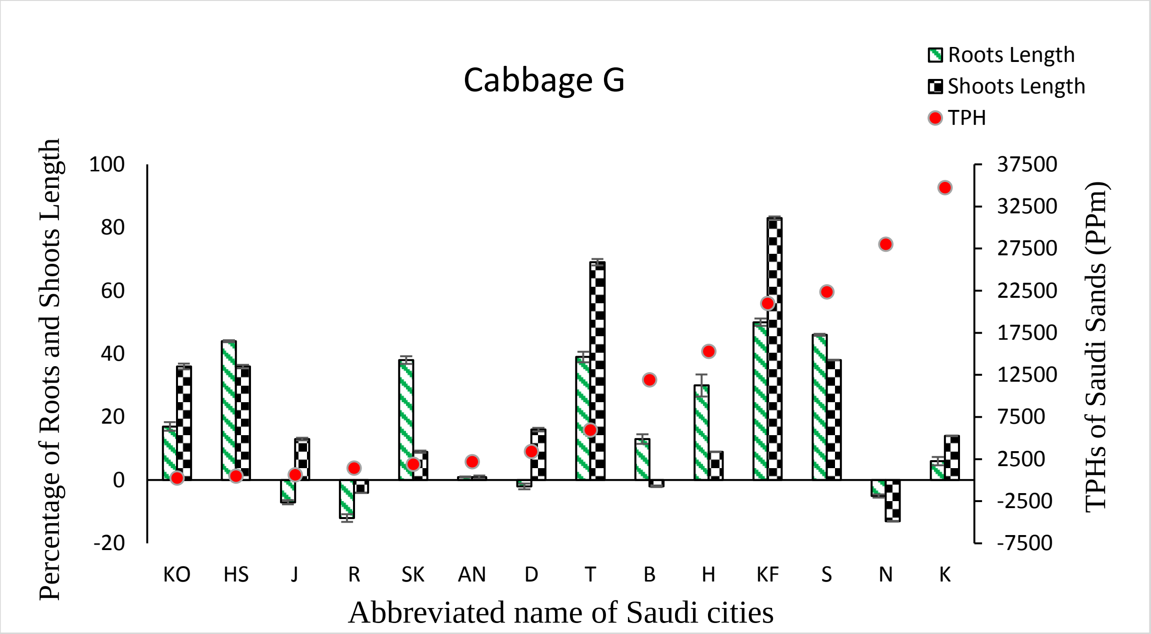


Figure 40 Root and shoot length of Cabbage Greyhound seedlings grown in all Saudi sands compared to root and shoot length in the control sand. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Different from the previously mentioned seeds, Cabbage Greyhound appeared to have some correlation between TPHs concentration and the on root length for samples Buraidah, Hufuf, Qautif, Sulayyil, Najran and Karj. As TPH content increased from 1.25% in Buraidah to 2% in Qatif, so did root length increase. At TPH content greater than 2%, Cabbage Greyhound root length decreased again. Shoot length of Cabbage Greyhound showed the same tendency.

Similar to Cabbage Gryehound, Coriander root and shoot growth (**Figure 41**) in the Saudi sands differed significantly from growth seen in the crude oil contaminated sand (**Figure 34**). There was an increase in root length of 82% in the Najran sample. The largest decrease -24% in root length was in the Buraidah sample. The shoot length decreased in 8 of the 14 samples with the largest decrease -30% seen in the Rafha sample and the greatest increase +67% seen in the Najran sample.

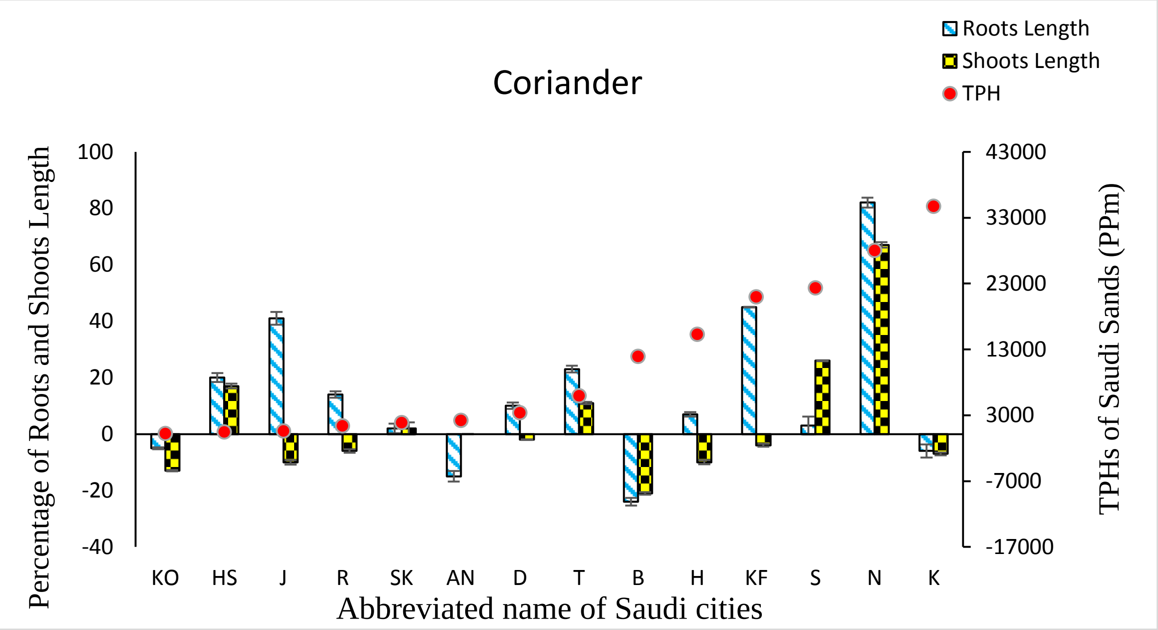


Figure 41 Root and shoot length of Coriander seedlings grown in all Saudi sands compared to root and shoot length in the control sand. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Again, there was no linear correlation between the TPHs and the root and shoot length for Coriander seedlings (**Figure 41**).

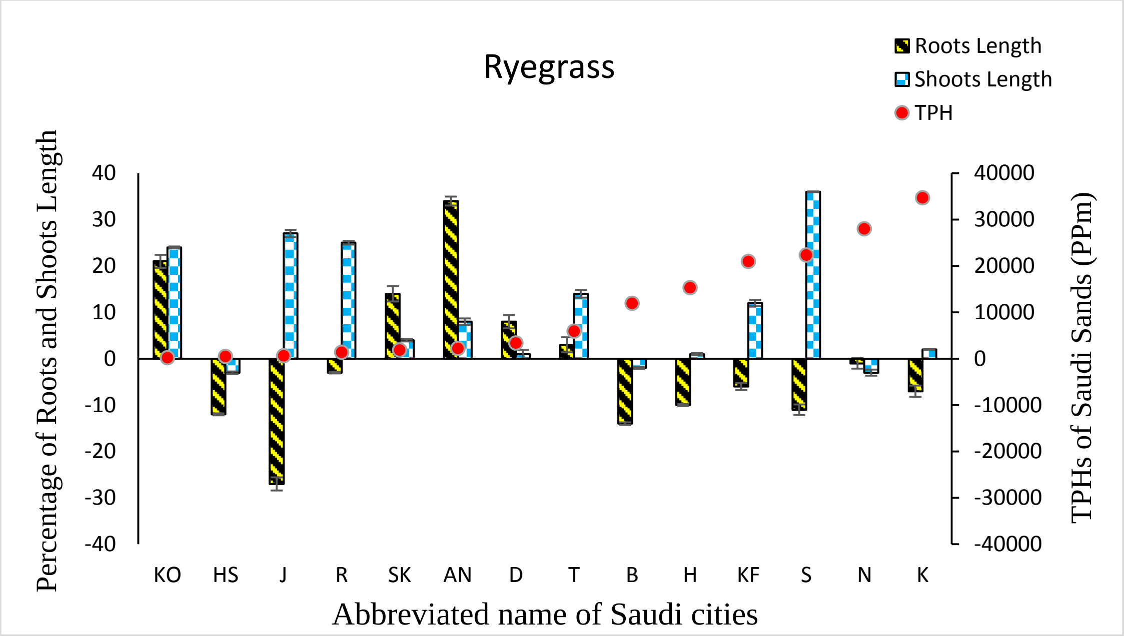


Figure 42 Root and shoot length of Ryegrass seedlings grown in all Saudi sands compared to root and shoot length in the control sand. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Figure 42 illustrates the root and shoot length for Ryegrass seedlings grown in Saudi sands. A large variation in root length was seen with the greatest decrease -27% seen in sample Jeddah and the largest increase +34% seen in sample Unaizah. Shoot length also varied from -6% in sample Rafha to an increase of 36% in sample Sulayyil compared to the control sand.

Contrary to the results seen in **Figure 34** in which Ryegrass root length increased up to a crude oil concentration of 2% and only decreased at contamination levels above 5%, Ryegrass root length decreased in all Saudi sands containing more than 0.5% TPHs (**Figure 42**). Shoot length was greatest in the Karj sand with TPHs concentration of 34708 mg/kg which is again different from the crude oil results.

Lastly, Wheat seedling growth also varied between all Saudi sand samples (**Figure 43**). The root length decreased by 23% in sample Hufuf and increased by 88% in sample Khafji, while the shoot length decreased by 18% in sample Hufuf and increased by 22% in sample Unaizah. Again, there was no linear correlation between TPH levels in the Saudi sands, root and shoot length. Root length generally increased compared to the control sand, like the results seen in the crude oil contaminated sand.

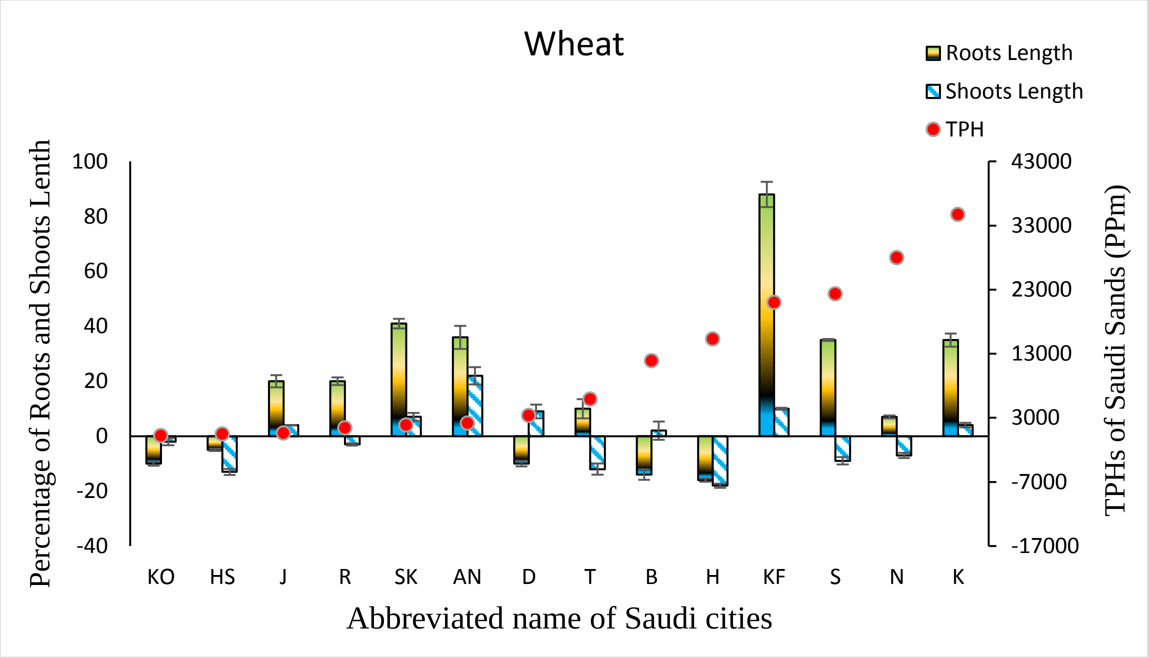


Figure 43 Root and shoot length of Ryegrass seedlings grown in all Saudi sands compared to root and shoot length in the control sand. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

The only direct similarity between Wheat growth in the crude oil contaminated sand (**Figure 34**) and the Saudi sands was that the greatest increase in root length seen here was in the Khafji sample containing TPHs concentration of 20995 mg/kg, which is equivalent to the 2% contaminated soil in **Figure 34**.

3.3.5 TPHs Yield Extracted from Contaminated CS Using Soxhlet and 24h Shaking.

TPHs from contaminated control sand was extracted using two methods a) a 3h Soxhlet extraction and b) 24h mechanical shaking. Both methods used the same solvent, DCM. **Figure 44 and 45** show the percentage of TPHs extracted from sand after crude oil contamination.

As seen from **Figure 44**, the greatest recovery seen from the Soxhlet extraction (using DCM as a solvent) was at 1% crude oil contamination (60.7% of the TPH) and the lowest recovery at 10% contaminated sand (24.2% of TPHs). Therefore, percentage recovery decreased with an increase in crude oil contamination.

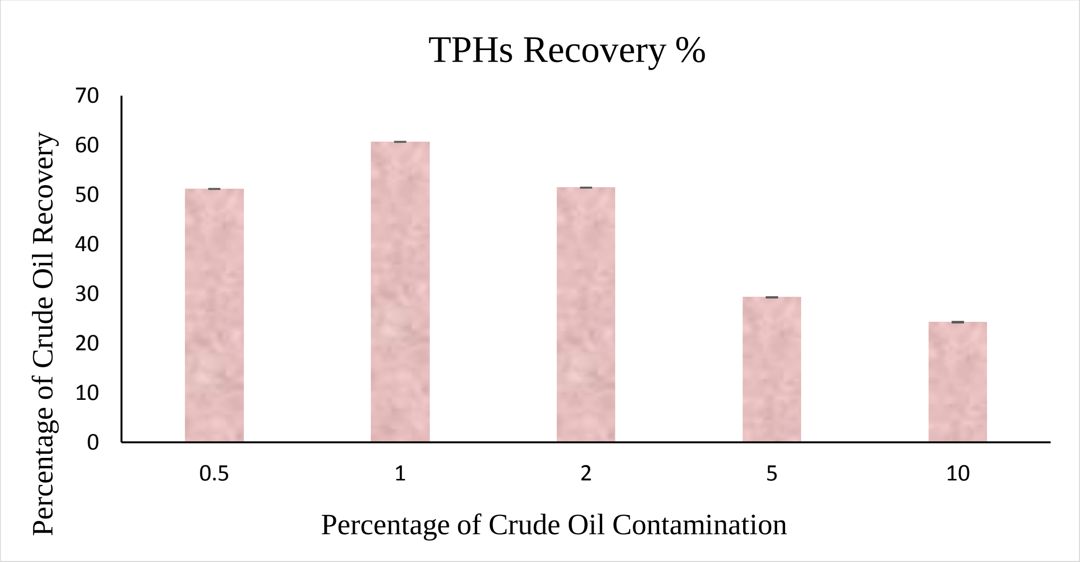


Figure 44 The percentage recovery of TPHs from crude oil contaminated sand using the Soxhlet extraction method. Bars indicate SEM of three replicates.

The 24 h mechanical shaking extraction method (**Figure 45**), showed the same trend as the Soxlet extraction (**Figure 44**). The largest extraction of TPH (19.8%) was at 0.5% and 1% crude oil contaminated sand and the lowest percentage extraction (8.97%) was at 10% contaminated sand.

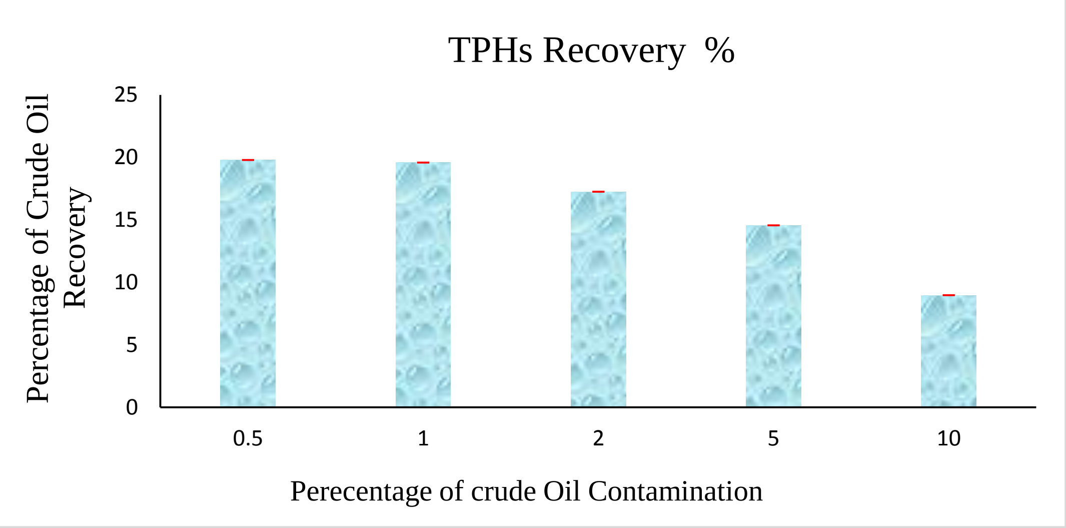


Figure 45 The percentage recovery of TPHs from crude oil contaminated sand using the 24hour extraction method. Bars indicate SEM of three replicates.

3.3.6 TPHs Yield after Seeds Germination Processes in Contaminated CS.

After harvesting the plants germinated in the contaminated sand in section 3.3.2, the contaminated sands were examined for TPH using Soxhlet and 24 h shaking extraction methods. The main aim of this investigation was to find out how much TPHs was taken up by the plants during germination and how much TPHs remained in the sand.

As seen from **Figure 46**, TPH were recovered from the contaminated sand after Radish germination. The percentage TPHs recovered using the 24 h shaking method apparently gave greater recovery compared to the Soxhlet method. The percentage of TPH recovered using the shaking method was above 100% for all contaminated sands evaluated. The highest percentage of TPH recovered from radish-germinated sand was at a 0.5% level of contamination (202%), followed by the 10% contaminated sand (156.7%). Soxhlet extraction gave significantly lower TPH results after Radish germination. The greatest percentage recovery by Soxhlet extraction was found for the 10% contaminated sand (42.7%) and the lowest recovery was found at 1% contamination (14.6%). The shaking method recovered more than the Soxhlet method in this experiment.

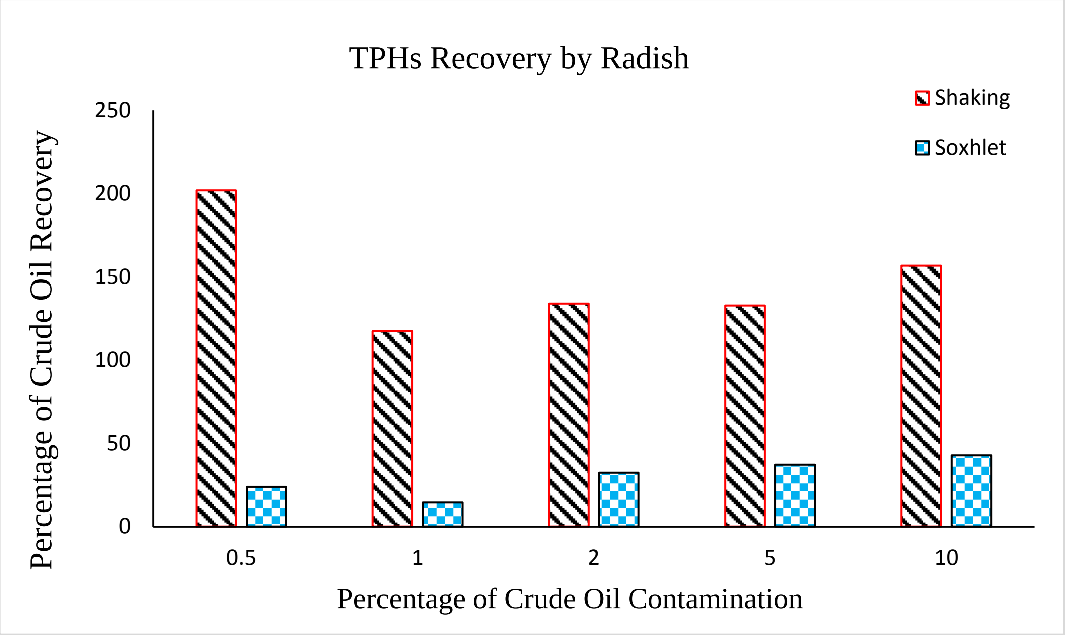


Figure 46 Percentage recovery of TPHs after Radish germination in contaminated sand using the 24 h shaking and Soxhlet extraction methods.

Next, TPHs were recovered after Lettuce germination in various crude oil contaminated sands using both extraction methods (**Figure 47**).

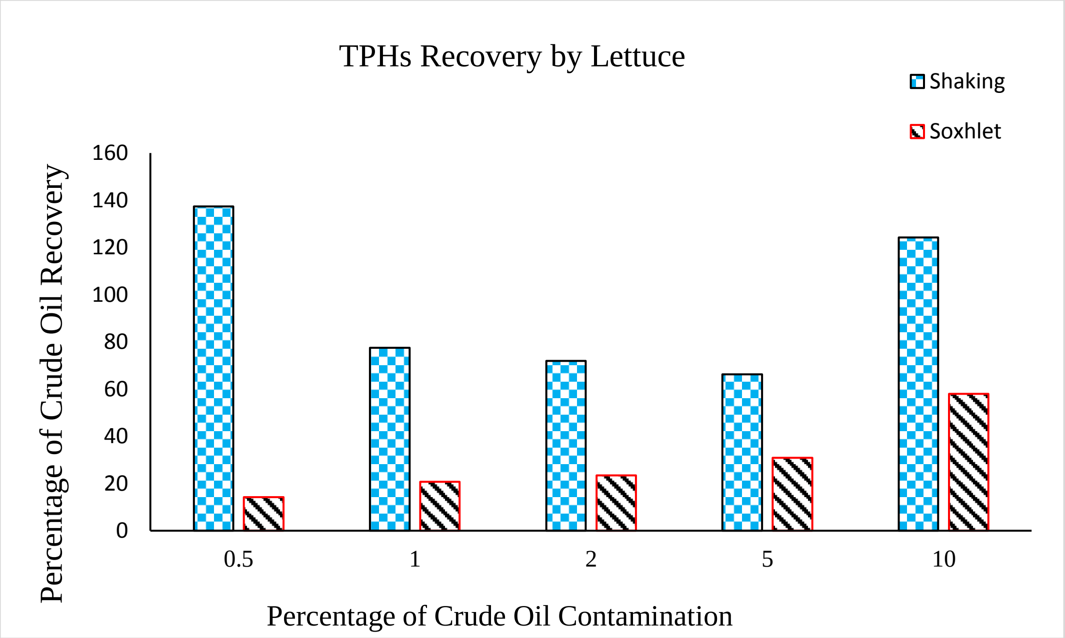


Figure 47 Percentage recovery of TPHs after Lettuce germination in contaminated sand using the 24 h shaking and Soxhlet extraction methods.

Again, the highest percentage TPH recovery was seen using the 24 h shaking method. The greatest recovery was for the 0.5% contaminated sand (137.4%) followed by the 10% contaminated sand (124.2%). The lowest percentage recovery was at a contamination level of 5% (66.2%). The Soxhlet method yielded lower TPHs. The highest percentage achieved with Soxhlet was 58% for a 10% crude oil contamination. The lowest TPH recovery was found at 0.5% (14.3%), followed by the 1% level of contamination (20.8%).

Next, TPHs were extracted from crude oil contaminated sand after Cabbage Red Drumhead germination (**Figure 48**). For Cabbage Red Drumhead, 24h shaking of contaminated sand also yielded more TPHs compared to Soxhlet extraction. The greatest recovery of TPH was seen at 0.5% contamination which decreased to 2% and increased again up to 10%. However, using Soxhlet extraction, there was a clear increase in TPHs recovery from 0.5% up to 10% contamination levels.

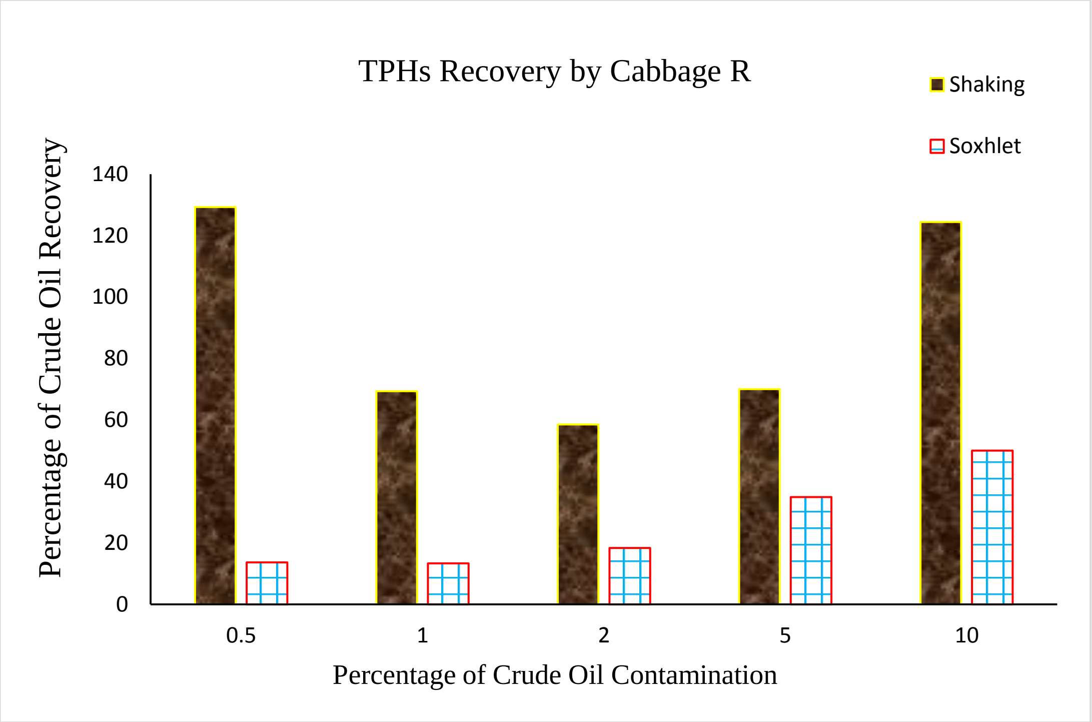


Figure 48 Percentage recovery of TPHs after Cabbage Red Drumhead germination in contaminated sand using the 24 h shaking and Soxhlet extraction methods.

Interestingly, the results for Cabbage Greyhound was different than that seen for Radish, Lettuce and Cabbage Red Drumhead as shown in **Figure 49**. Using 24 h shaking, there was an increase in TPH extracted from 0.5% up to 10% contamination levels, apart from 2% which decreased slightly. The recovery of TPHs, using Soxhlet extraction after Cabbage Greyhound germination, gradually increased from 0.5% to 10% contaminated sands. The highest yield of TPHs was at 1% contaminated sand (**Figure 49**). Soxhlet extraction for the other seed types gave the highest recovery at 10% crude oil contamination.

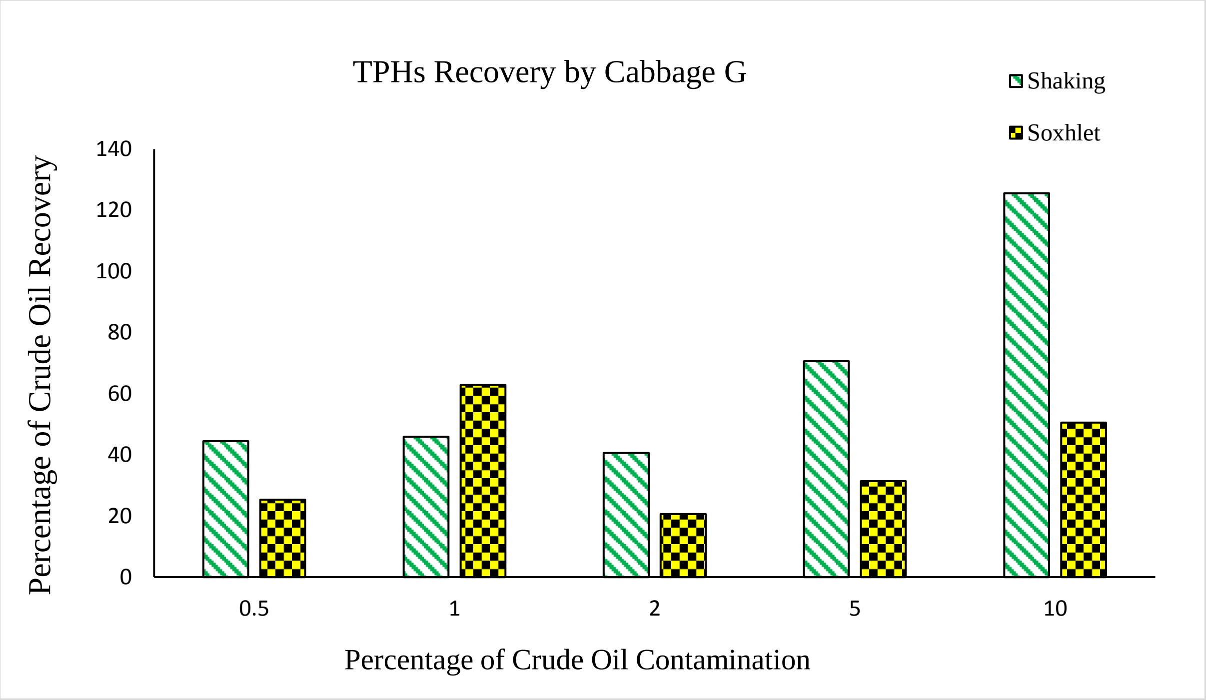


Figure 49 Percentage recovery of TPHs after Cabbage Red Drumhead germination in contaminated sand using the 24 h shaking and Soxhlet extraction methods.

Figure 50 shows the percentage recovery of TPHs from sand in which coriander was germinated. There was an increase in TPH recovery from 1% to 10% contaminated sand using 24 h shaking, and the recovery was equal for the 0.5% and 5% contaminated sands. At the 10% contamination level, the recovery was 118% compared to control sand and the lowest recovery was found at 1%. To the contrary, the highest TPHs recovery using the Soxhlet extraction experiment was found at 1% followed by 10%, 5%, 2% and 0.5% contamination levels.

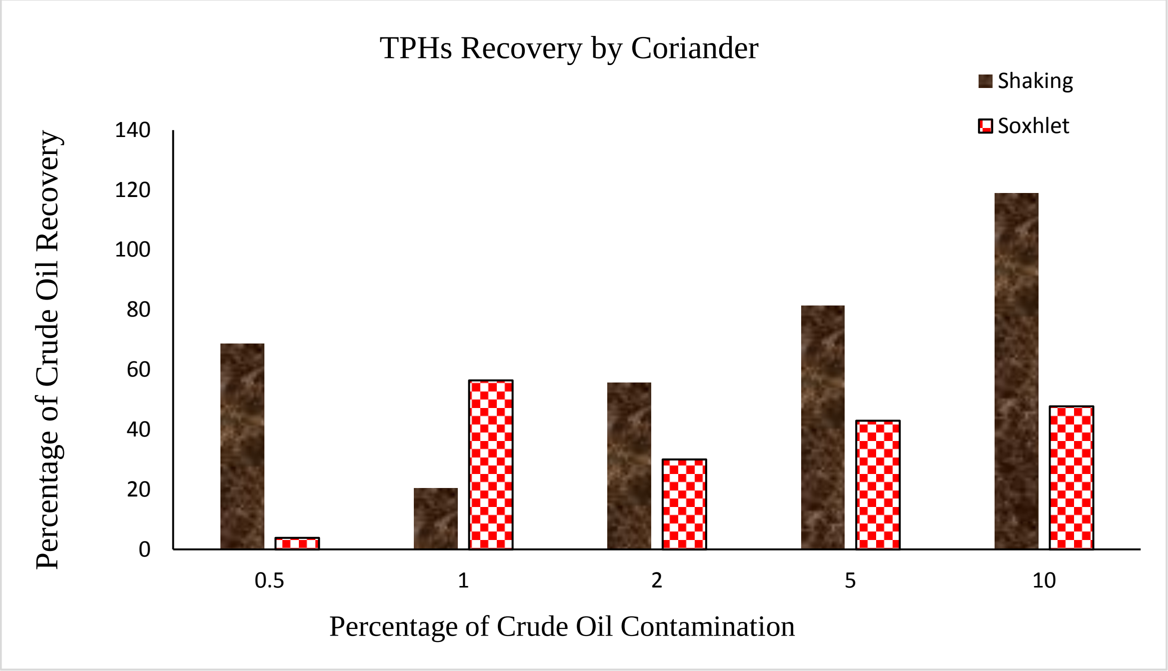


Figure 50 Percentage recovery of TPHs after Coriander germination in contaminated sand using the 24 h shaking and Soxhlet extraction methods.

After Ryegrass germination (**Figure 51**), the percentage THP recovery for both the 24hour and Soxhlet extraction methods followed the same trend. The extraction method gave a clear increase in the TPHs recovery from 0.5% up to 10% crude oil contaminated sand.

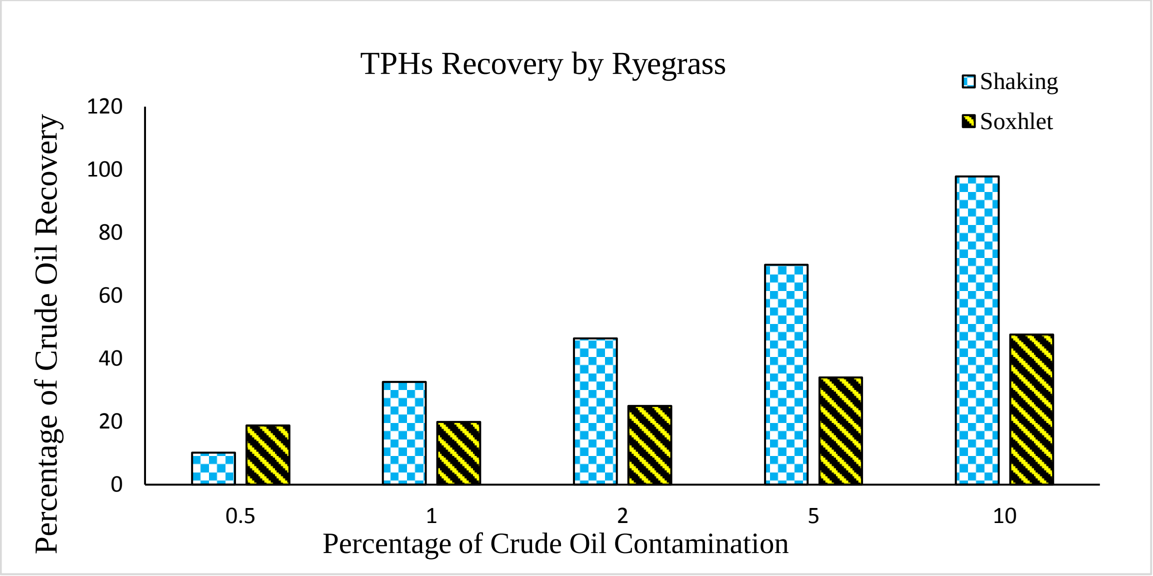


Figure 51 Percentage recovery of TPHs after Ryegrass germination in contaminated sand using the 24 h shaking and Soxhlet extraction methods.

Different from most other seedlings, there was an overall decrease in the TPHs recovery in sand after Wheat germination using the 24hour extraction method (**Figure 52**). The Soxhlet extraction method gave similar results, except at 10% contamination the percentage recovery was greater than at 1% contamination.

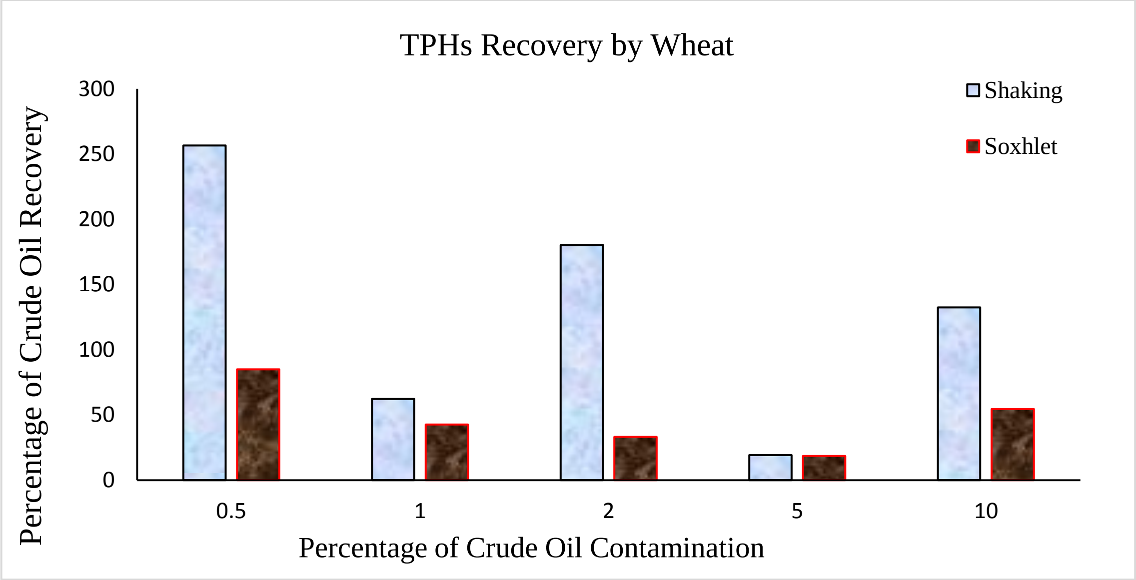


Figure 52 Percentage recovery of TPHs after Wheat germination in contaminated sand using the 24 h shaking and Soxhlet extraction methods.

3.3.7 Seeds Germination Conducted in Saudi Arabia.

The seed germination and seedling growth experiments conducted in the UK, were next repeated in KSA. The seven types of plants were planted in all types of sand (contaminated, uncontaminated CS and Saudi sands) and conducted in KSA in order to determine the effect of climate conditions on growth rate of seeds. The root and shoot lengths were measured and compared with similar experiments that were conducted in the UK.

3.3.8 Seeds Germination Conducted Outdoors in KSA.

Firstly, seven types of plants were planted in contaminated and uncontaminated control sand in KSA. These samples were put in petri dishes on a table outdoors for two weeks without controlling the conditions (temperature, humidity and day light hours). Samples were watered twice a day (5 ml). No germination was observed in any of the samples after two weeks (**Figure 53** and **Figure 54**), which was thought to be linked to the high heat and the direct sunlight. The temperature in KSA (Riyadh city) was approximately 48°C during the experiment which could have prevented germination.

Figure 53 Image showing no germination activity of seeds outdoors in contaminated and uncontaminated CS.

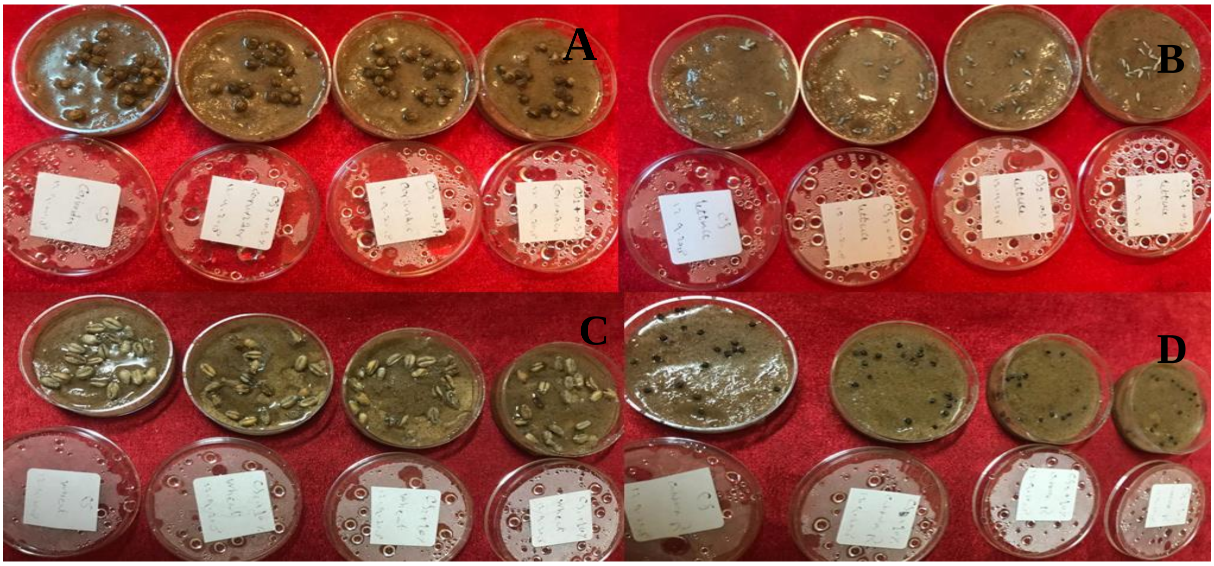


Figure 54 Image showing no germination activity of Coriander (A), Ryegrass (B), Wheat (C) and Cabbage Red (D) after two weeks outdoors in KSA.

3.3.9 Seeds Germination Conducted Indoors without Air-Conditioning in KSA.

Next it was decided to repeat the germination experiments indoors to avoid the high temperatures and direct exposure to sunlight. After one week some germination was observed in some plants (**Figure 55**). The germination percentage was however not as good as for experiments conducted under controlled conditions in the UK.

Figure 55 Seven types of seeds after two weeks in indoors in KSA, showing no germination activity.

3.3.10 Seeds Germination Conducted Indoors with 12 hours Air-conditioning in KSA.

Following on from the previous two experiments an experiment was conducted using an air-conditioned room (12 hours) for two weeks (**Figure 56**). Germination was better than in the previous experiments, but still not enough to be harvested and some sand samples had no germination.

3.3.11 Seeds Germination Conducted Indoors with 24 hour Air-conditioning in KSA.

From the three previous experiments, it became apparent that the seed germination experiments needed to be repeated in an air-conditioned room in order to avoid the high heat, the exposure of sunlight and to get better germination percentages. Healthy growth was observed for most seeds after five days (**Figure 57**). Therefore, this experiment was applied to all contaminated, uncontaminated control sand and Saudi sands. The root and shoot length was measured for all samples after harvesting.

Figure 56 Germination activity of seven types of seeds after two weeks indoors in KSA with 12 hours air-conditioning.

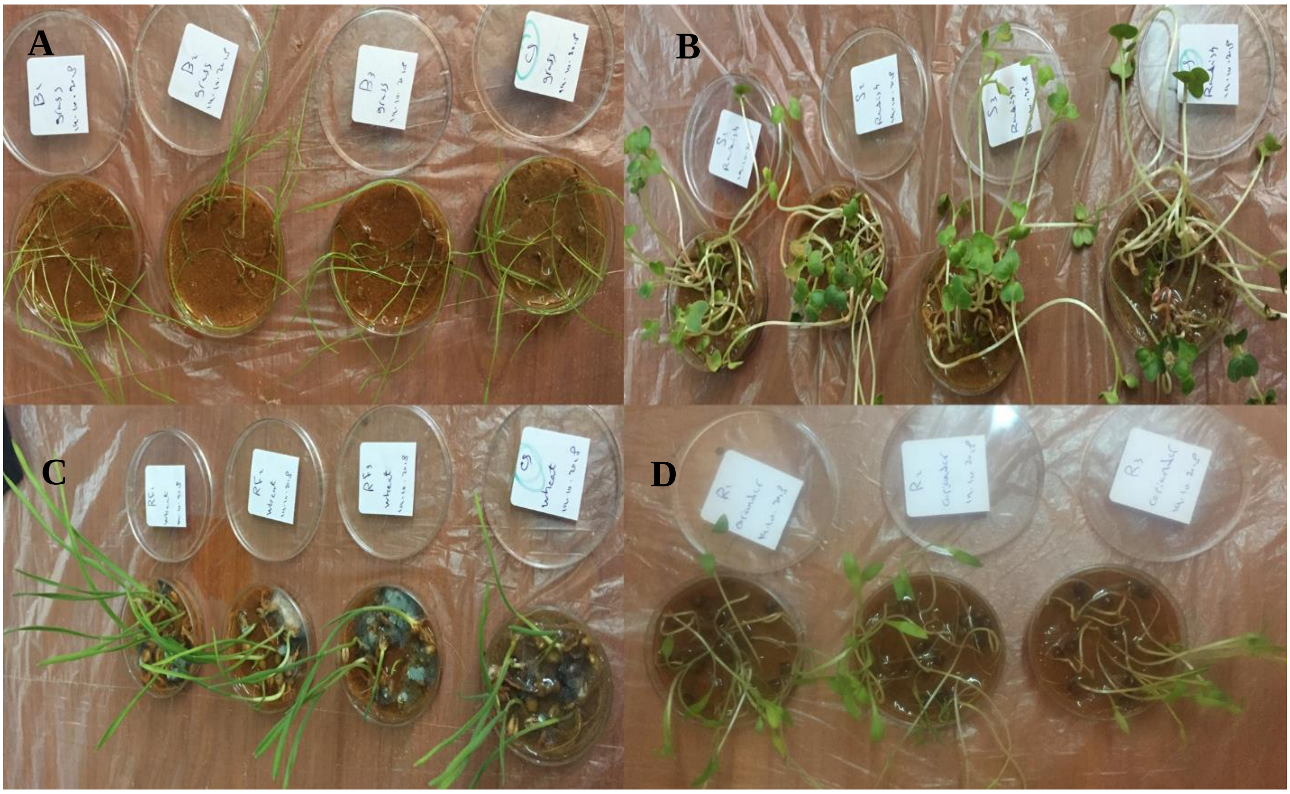


Figure 57 Germination activity of Ryegrass (A), Radish (B), Wheat (C) and Coriander (D) seeds after two weeks indoors and 24 h air-conditioning in KSA.

3.3.12 Percentage Germination in Control and Contaminated Sands Conducted in KSA.

Seven different seed types were planted in petri dishes containing control sand or sand contaminated with 0.5%, 1%, 2%, 5% and 10% crude oil. Growth conditions were kept the same for consistency (**Figure 58** and **59**).

Seeds were planted indoors in KSA with 24hours air-conditioning in control and contaminated sand (0.5% up to 10% crude oil, **Figure 60**). The germination percentage was calculated by counting the number of seeds that germinated from the 20 seeds that were initially planted. Germination was varied for all plants at different concentrations of crude oil. At 1% of contamination, Ryegrass and Lettuce had the highest percentage of germination with 92% and 90% respectively. At 5% contamination, Wheat had the lowest germination percentage among all samples (**Figure 60**).

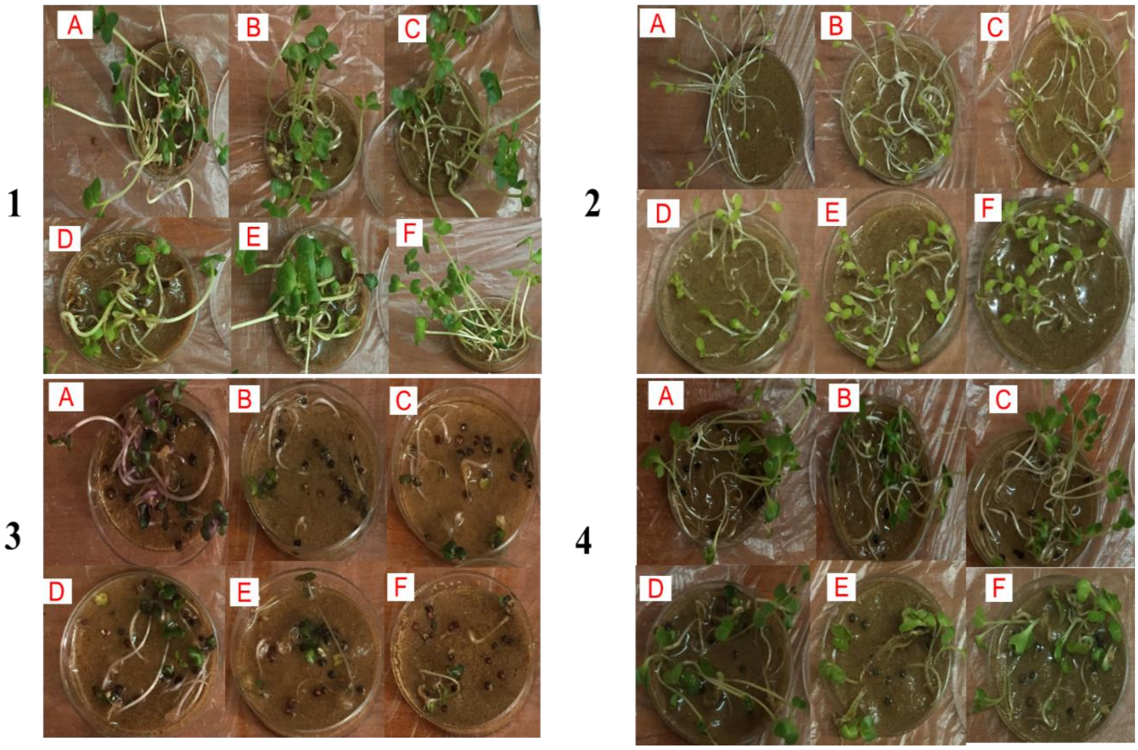


Figure 58 Plants germinated before harvesting Radish (1), Lettuce (2), Cabbage Red Drumhead (3) and Cabbage Greyhound (4) in CS (a), 0.5% (b), 1% (c), 2% (d), 5% (e) and 10% (f) of crude oil (from top left to right).

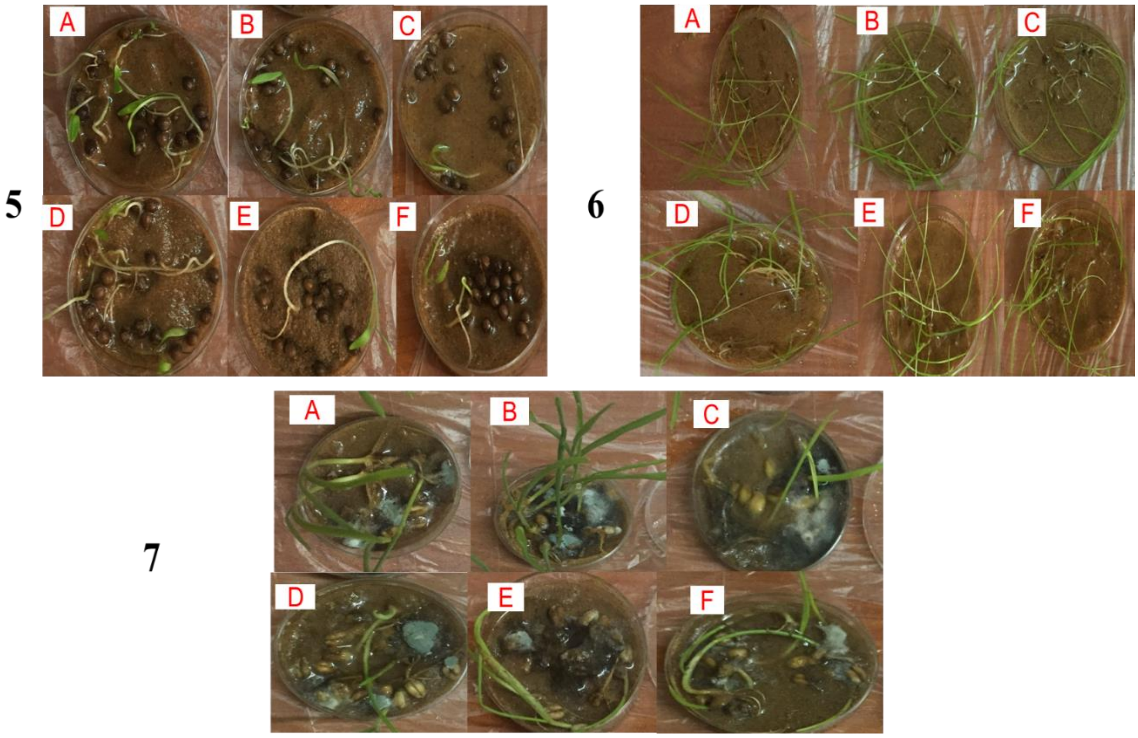


Figure 59 Plants germinated before harvesting Coriander (5), Ryegrass (6) and Wheat (7) in CS (a), 0.5% (b), 1% (c), 2% (d), 5% (e) and 10% (f) of crude oil (from top left to right).

The percentage germination for Radish ranged from 18% (at 2% crude oil) to 85% (at 0.5% crude oil), while the percentage of seeds germinating in control sands was 80% (**Figure 60**). For Lettuce, percentage germination ranged from 82% (at 0.5% crude oil) to 90% (at 10% crude oil), while the percentage of seed germination in the control sand was 80%. Cabbage Red Drumhead ranged from 8% (at 5% crude oil) to 18% (at 1% crude oil), while the percentage of seed germination in the control sand was 20%. Cabbage Greyhound ranged from 40% (at 10% crude oil) to 73% (at 0.5% crude oil), while the percentage of seed germination in the control sand found to be 73%. Coriander ranged from 7% (at 5% crude oil) to 13% (at 0.5% crude oil), while the percentage of seed germination in the control sand was 13%. Ryegrass ranged from 68% (at 5% crude oil) to 90% (at 2% crude oil), while the percentage of seed germination in control sand was 91%. Wheat ranged from 5% (at 5% crude oil) to 33% (at 0.5% crude oil), while the percentage of seed germination in the control sand was 22%.

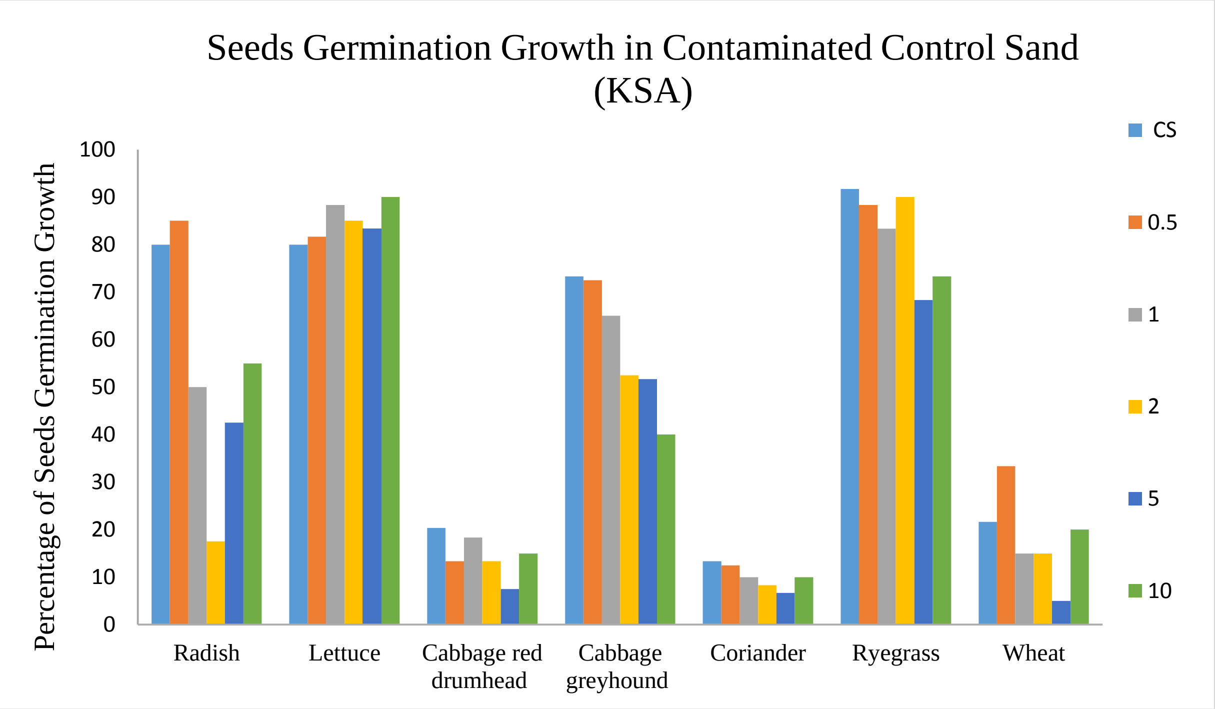


Figure 60 Percentage seed germination for control and contaminated sand containing 0.5, 1, 2, 5 and 10% crude oil. Experiments were conducted in KSA.

3.3.13 Seedling Growth in Uncontaminated and Crude Oil Contaminated sand in KSA.

Radish planted in contaminated control sand had a negative response at most concentrations of crude oil contamination (**Figure 61**). There was an increase in root length (+25%) at 0.5% of crude oil, then a clear decrease (up to -46%) from 1% to 10% of crude oil. Regarding the shoot length, there was a decrease (up to -45%) from 0.5% up to 10% of crude oil apart from at 1%, which had a shoot length equal to the control sand.

Lettuce had an increase in root length at all levels of contamination **(Figure 61**) with the longest roots found at 2% crude oil. Shoot length only became affected at crude oil levels at and above 2% and decreased by 14% at 10% crude oil contamination.

Figure 61 shows that Cabbage Red Drumhead planted in contaminated sand was negatively affected at all levels of crude oil. The greatest decrease (-38%) in root length was found at 2% crude oil. Shoot length also decreased the most at 2% contamination (-10%).

Cabbage Greyhound planted in a contaminated sand and grown in KSA also had a negative response at all crude oil levels (**Figure 61**).The shortest roots were found at 0.5% (-19%) while 10% contamination appeared to have no effect on root length. The shortest shoots were found at 10% crude oil (-33%).

Different from the above-mentioned seeds, Coriander had positive responses at all levels of contamination (**Figure 61**). The longest roots were found at 0.5% crude oil (+41%) followed by 2% crude oil (+27%). Shoot length appeared to be unaffected at 2% crude oil levels but increased at all other levels. The longest shoots were found at 1% crude oil (+17%).

Figure 61 and **62** show that the *p* value of all plants had been analysed for roots and shoots of control sand and all level of contamination of control sand compared to each other. The result (**Figure 61**) for radish showed that there was no significant difference between roots at 1 % and 5 % ($p < 005$) compared to all control and contaminated sand, also there was no significant difference between 1 % and 5 % as the *p* value < 0.05. In addition, there was no significant difference between shoots at 0.5 % , 1 %, 2 %, 5 % and 10 % ($p < 005$). However, there was a significant diffeernce between shoots at 2, 5 and 10 % compared to control sand and all levels of contamination apart from at 0.5 and 1 %.

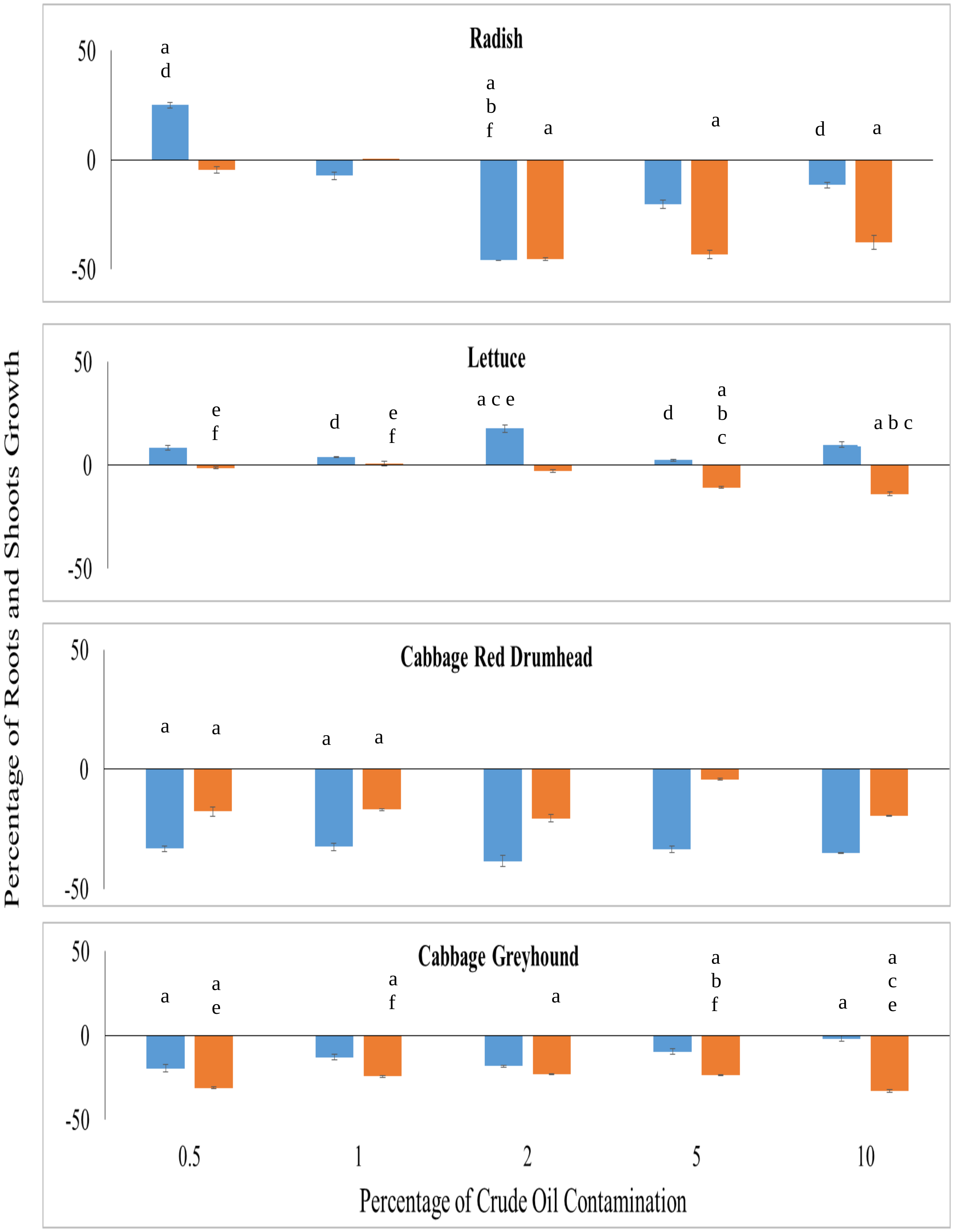


Figure 61 Response in seeds germination conducted in KSA indoors 24h air conditioner of radish, lettuce, cabbage red drumhead and cabbage greyhound with roots (blue) and shoots (orange) in a control sand contaminated with crude oil. Bars error bars indicate SEM of three replicates. Where the alphabet, a (control sand), b (0.5 %), c (1 %), d (2 %), e (5 %) and f (10 %) represent a significant difference ($p < 0.05$) in mean values ($n = 3$).

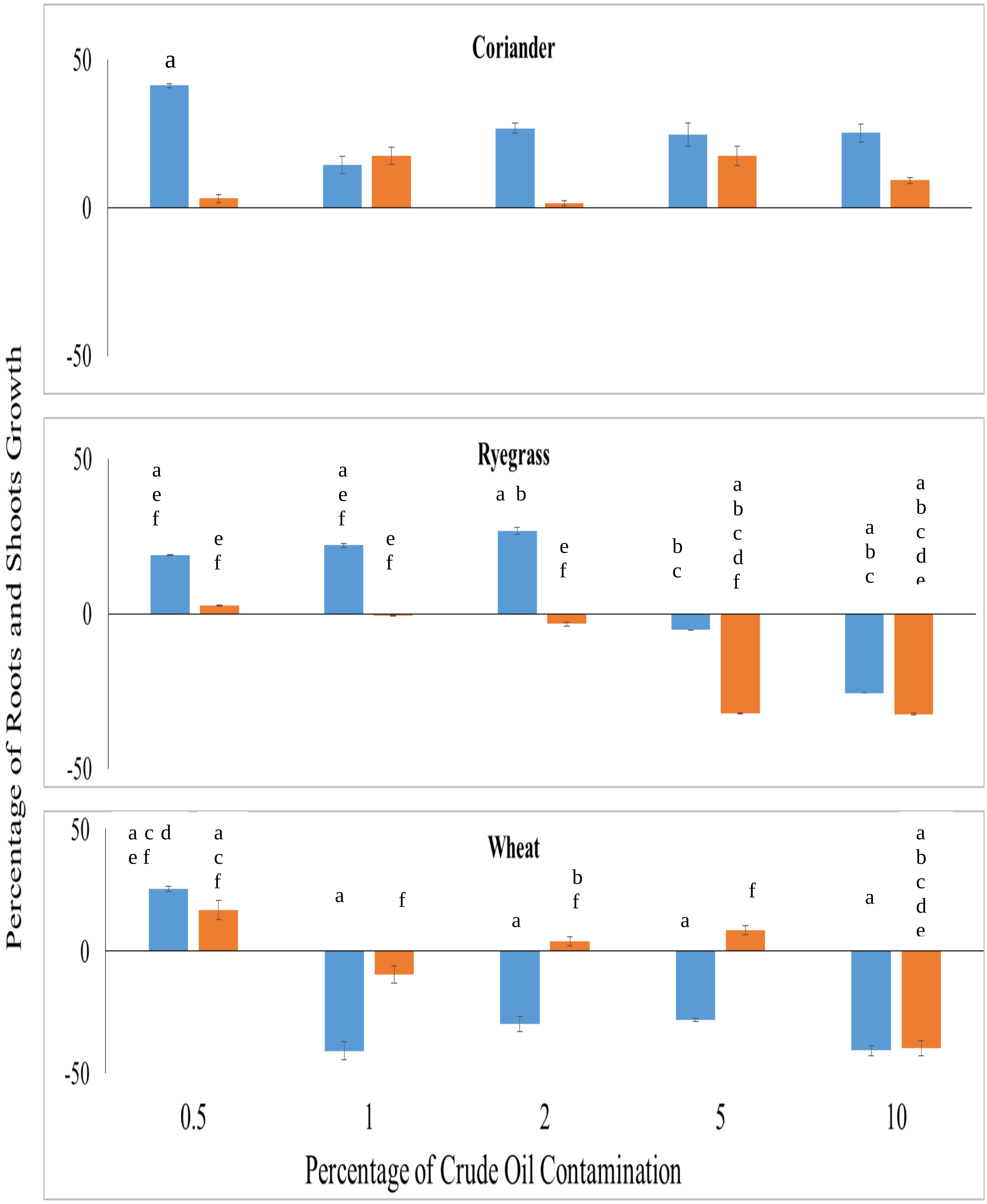


Figure 62 Response in seeds germination conducted in KSA indoors 24h air conditioner of coriander, ryegrass and wheat with roots (blue) and shoots (orange) in a control sand contaminated with crude oil. Bars error bars indicate SEM of three replicates. Where the alphabet, a (control sand), b (0.5 %), c (1 %), d (2 %), e (5 %) and f (10 %) represent a significant difference ($p < 0.05$) in mean values ($n = 3$).

Ryegrass planted in contaminated sand had variable responses (**Figure 61**). There was an increase in root length from 0.5% up to 2% of crude oil followed by a clear decrease in root length from 5% to 10% of crude oil. Shoot length appeared unaffected up to contamination levels of 2%, after which shoot length also decreased by up 33% at 10% crude oil.

The results for Wheat planted in contaminated sand are illustrated in **Figure 61**. Wheat had shorter roots than in control sand, apart from the 0.5% crude oil sample which had an increase of root length (+26%). The shortest roots were found at 2 and 10% crude oil (-41%). There was an increase (+17%) in shoot length at 0.5% contamination after which there was a decrease at all other levels (-40% at 10% crude oil).

Wheat and Coriander had very different responses in terms of root and shoot length compared to the UK results. Wheat in KSA had a negative response at all levels of contamination, except at 0.5%, whereas Wheat grown in the UK had positive responses at all contamination levels. Coriander grown in KSA responded positively compared to growth in the UK. It would thus appear that Coriander prefers the warmer climate of KSA. Radish, Lettuce, Cabbage Red Drumhead, Cabbage Greyhound and Ryegrass had a similar response in both countries in terms of root and shoot length (**Figure 61 and Figure 33**).

Taken together, for seeds grown in KSA Coriander was not susceptible to crude oil contamination. Ryegrass and Lettuce were positively affected up 2% contamination. Cabbage Red Drumhead, Cabbage Greyhound, Wheat and Radish were found to be very susceptible to the conditions in the KSA crude oil experiment.

3.3.14 Seedling Growth for All Saudi Sand Samples Conducted in KSA.

It was noticed that there was a variation in seedling growth in all Saudi sands compared to the control sand, as shown in the following figures (**Figure 63-69**).

Radish grown in Saudi sands varied greatly regarding root and shoot length (**Figure 63**). The shortest roots were found in sample S (-5%), while the longest roots were found in sample Dammam (+76%). The longest shoots were found in sample Hufuf (+21%), while the shortest shoots were found in sample Riyadh (-45%). There appeared to be a correlation between root and shoot length and the concentration of TPHs in the Saudi sands. The best growth was seen for sample Dammam which contained a concentration of TPH below 0.5% crude oil. When the concentration of TPHs increased above 0.5% of crude oil, a negative effect was seen on Radish growth.

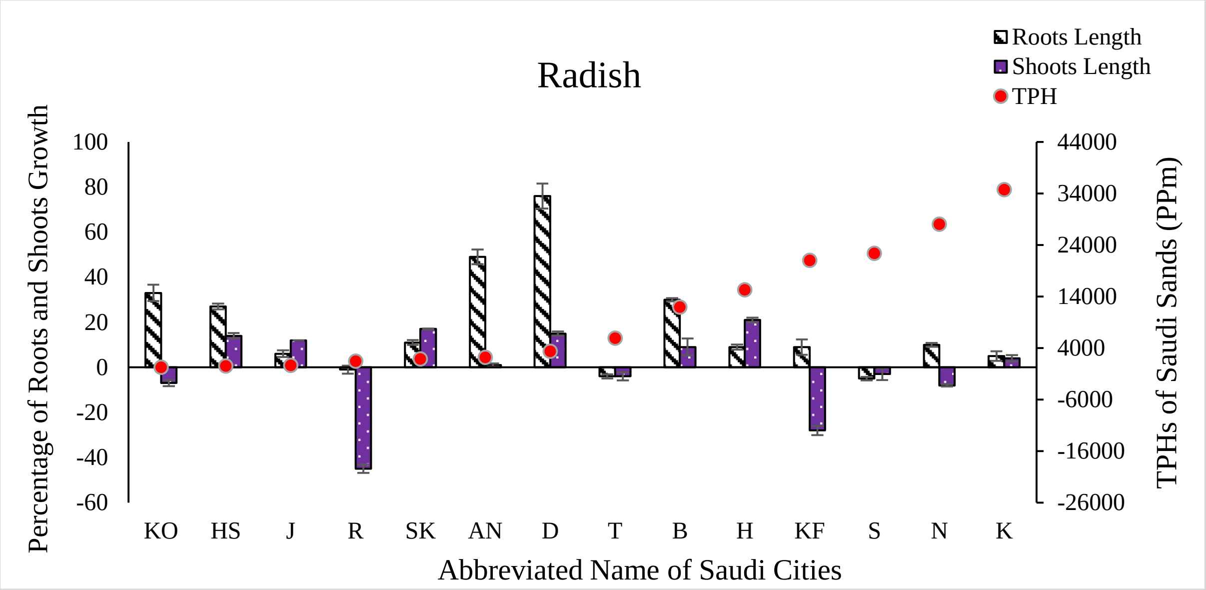


Figure 63 Comparison between radish root and shoot length in all Saudi sands. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Figure 64 shows the variation in root and shoot length for Lettuce grown in all Saudi sands. The root length ranged from -3% in sample Karj up to 37% in sample Unaizah. Shoots length ranged from -21% in sample Rafha to 20% in sample Al-Hassy. By comparing root and shoot length with concentration of TPHs, there was a clear decrease in growth in Saudi sands when the TPH concentration was equal or above to 1.5% crude oil contamination.

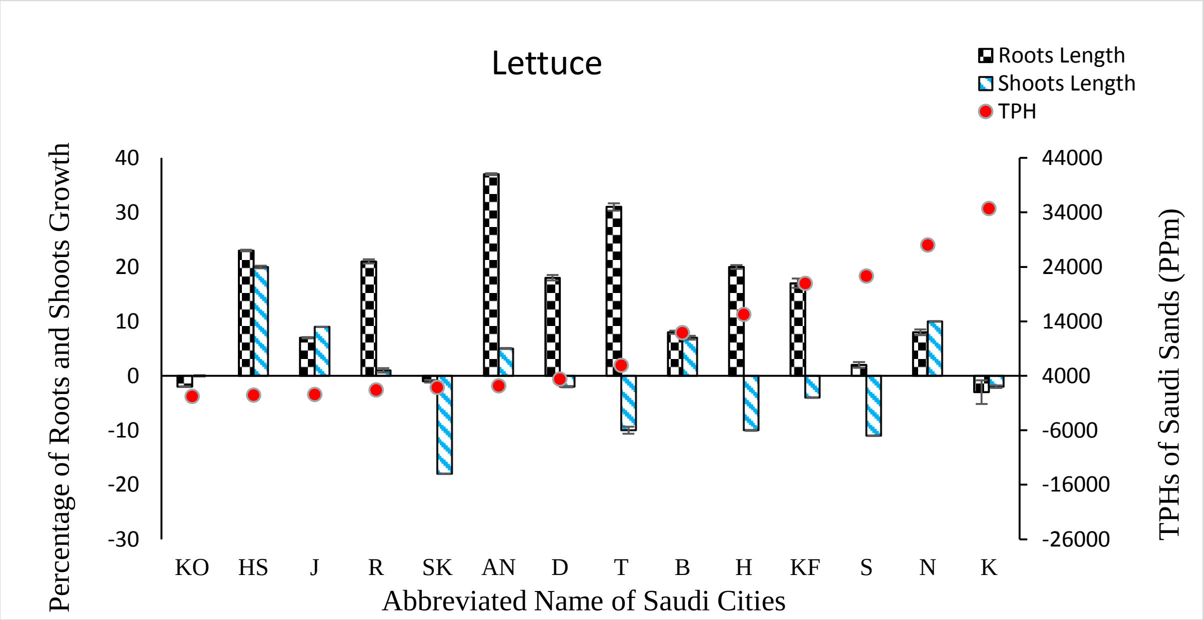


Figure 64 Comparison between Lettuce roots and shoots length in all Saudi sands. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Cabbage Red Drumhead was grown in all Saudi sand samples and again there was a variation in root and shoot length (**Figure 66)**. The roots length ranged from -35% to +23%, compared to control sand and the shortest roots were in sample Tabuk, while the longest roots were in sample Karj. Shoot length ranged from -57% in sample Dammam to +37% in sample Riyadh (R). Comparing concentration of TPHs of Saudi sands to seedling growth showed that Khafji, Sulayyil and Karj sands increased root and shoot length as TPHs increased.

In **Figure 66**, Cabbage Greyhound showed variation in growth between all Saudi sands. The root length decreased by 18% in sample Rafha and increased by 28% in sample Unaizah. Shoots length decreased by 36% in sample Khafji and increased by 28% in sample Unaizah. By increasing the concentration of TPHs of Saudi sands, there was a clear decrease in root and shoot growth in most samples. Based on these results it was concluded that Cabbage greyhound does not like to grow in a contaminated environment.

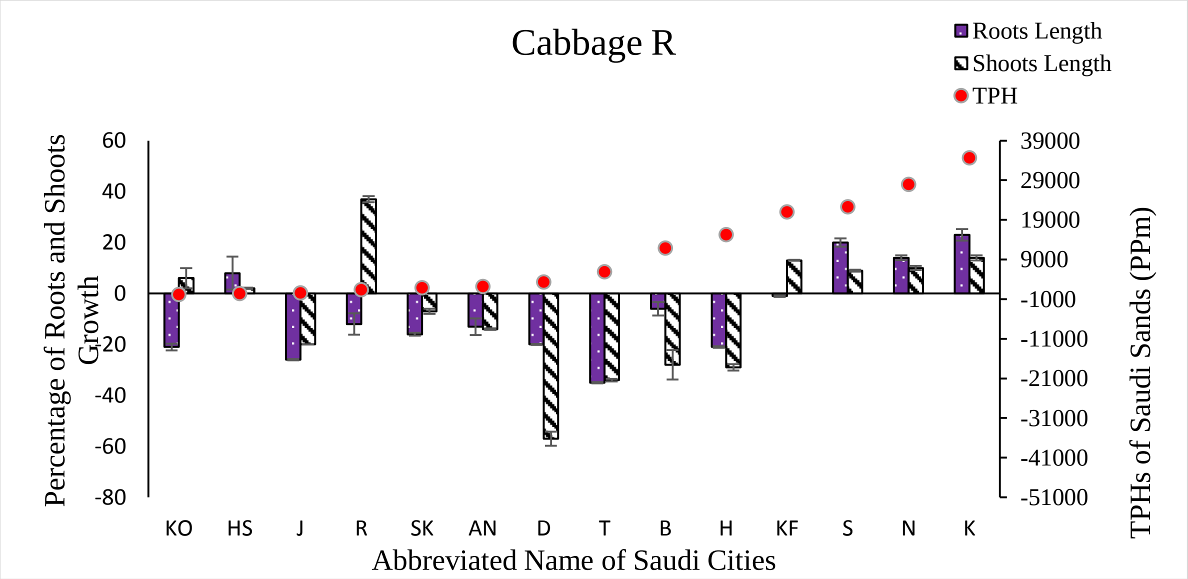


Figure 65 Comparison between Cabbage Red Drumhead root and shoot length in all Saudi sands. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

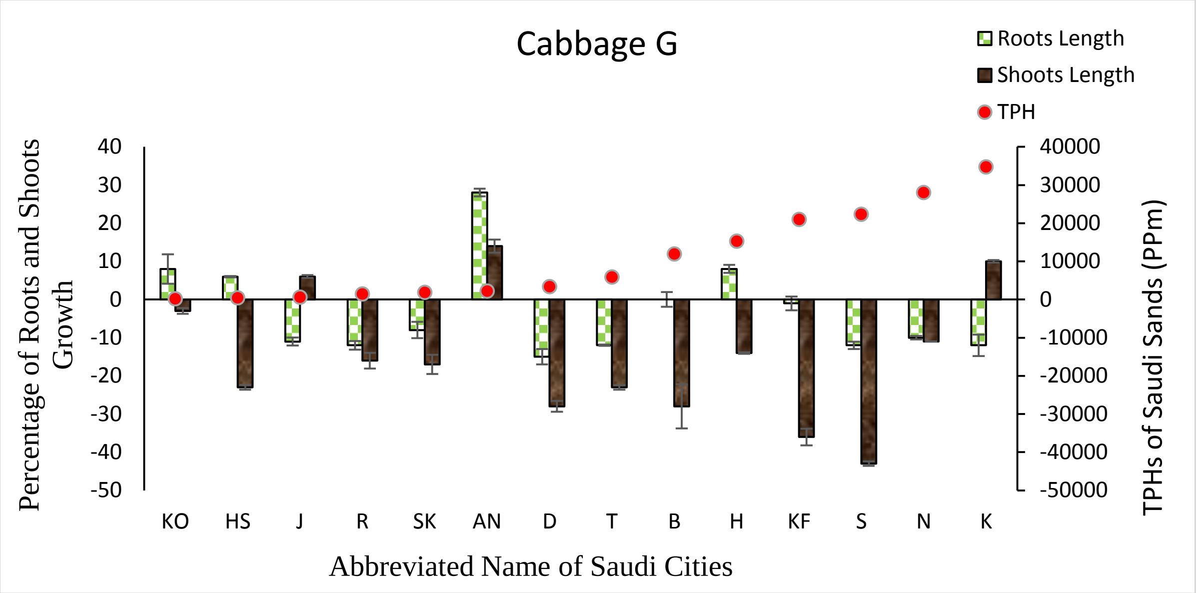


Figure 66 Comparison between Cabbage Greyhound root and shoot length in all Saudi sands. Bars indicate SEM of three replicates.

Results for Coriander grown in all Saudi sands are presented in **Figure 67**. The root length decreased by 18% in sample Dammam and increased by 79% in sample Unaizah. Shoot length ranged from -13% in sample Rafha up to +22% in sample Hufuf. It was noticed that at a concentration of TPHs correlating to 1% crude oil there was a decrease in root and shoot growth. Coriander does not appear to grow well in an environment containing more than 1% crude oil.

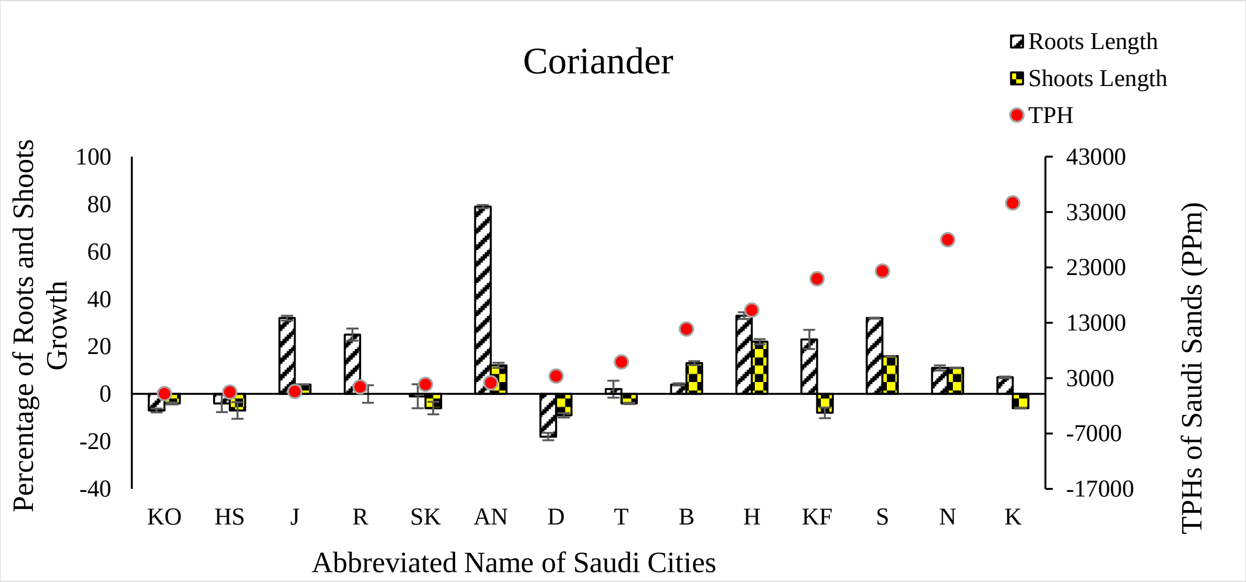


Figure 67 Comparison between Coriander root and shoot length in all Saudi sands. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

Figure 68 shows the results of root and shoot length for Ryegrass grown in all Saudi sands. The root length decreased by 30% in sample Riyadh and increased by 38% in sample Unaizah. Shoots length ranged from -11% in sample Riyadh and +15% in sample Buraidah. Increasing TPHs concentration appeared to cause a decrease in root length except for sample SK, Unaizah, Hufuf and Khafji.

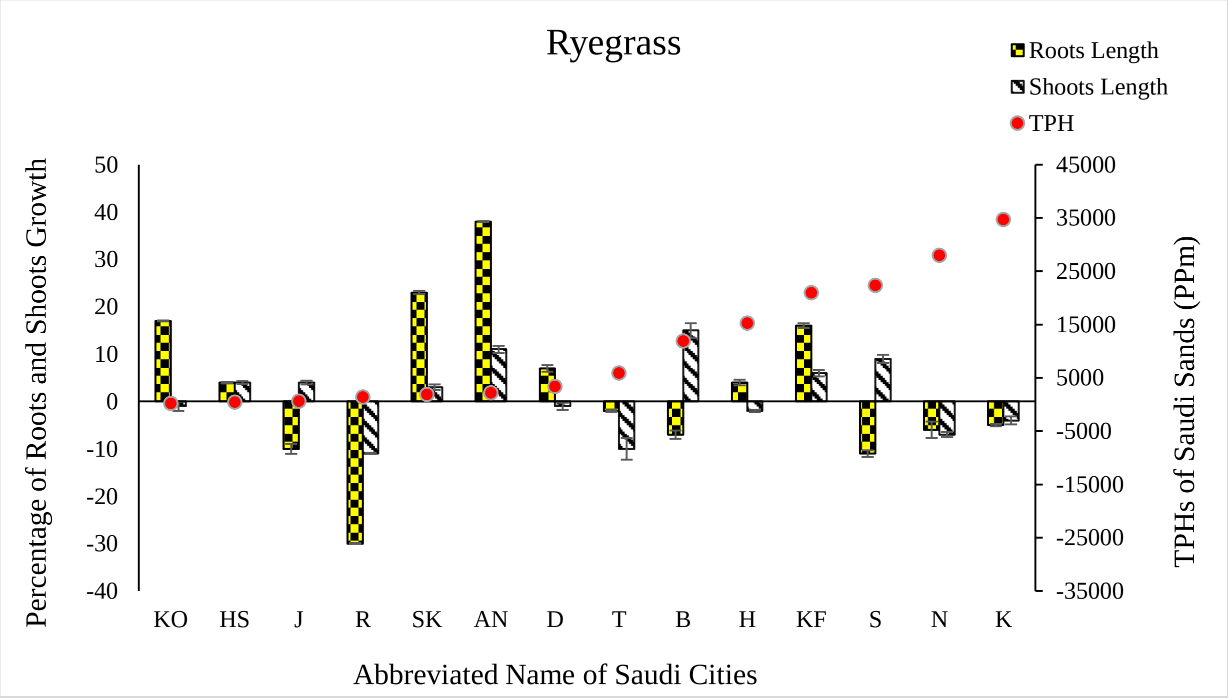


Figure 68 Comparison between Ryegrass root and shoot length in all Saudi sands. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

The results for Wheat grown in all Saudi sands are presented in **Figure 69**. The root length ranged from -10% in sample Qatif up to +118% in sample Unaizah. Shoot length decreased by 17% in sample Hufuf and increased by 86% in sample Unaizah. Comparing root and shoot length to concentration of TPHs, it was noticed that above 0.2% of crude oil there was a clear decrease of growth in Saudi sands.

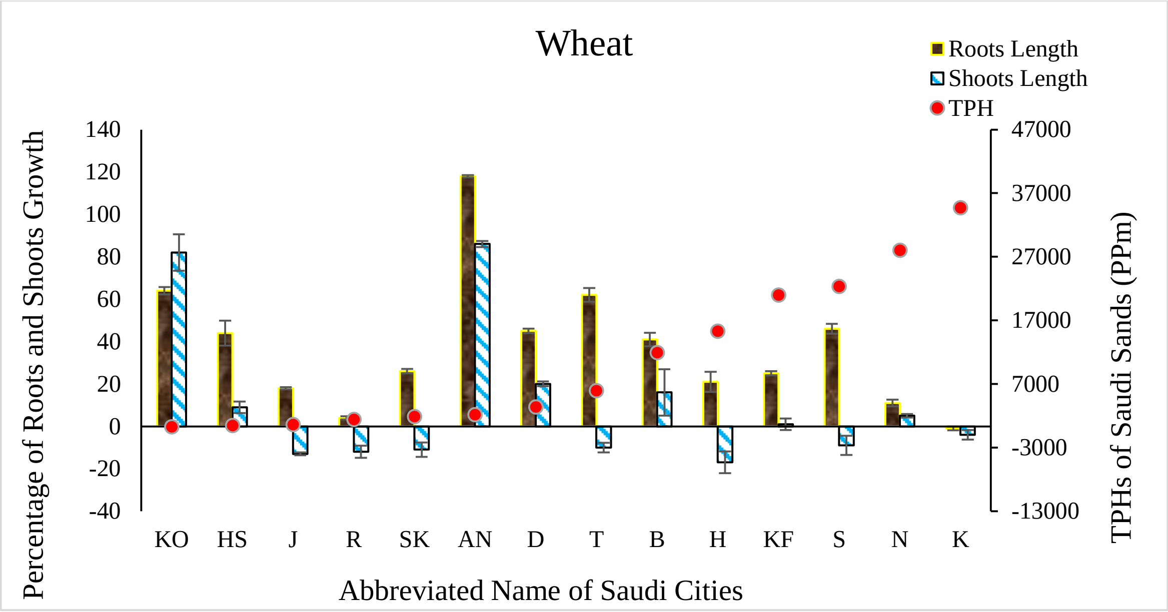
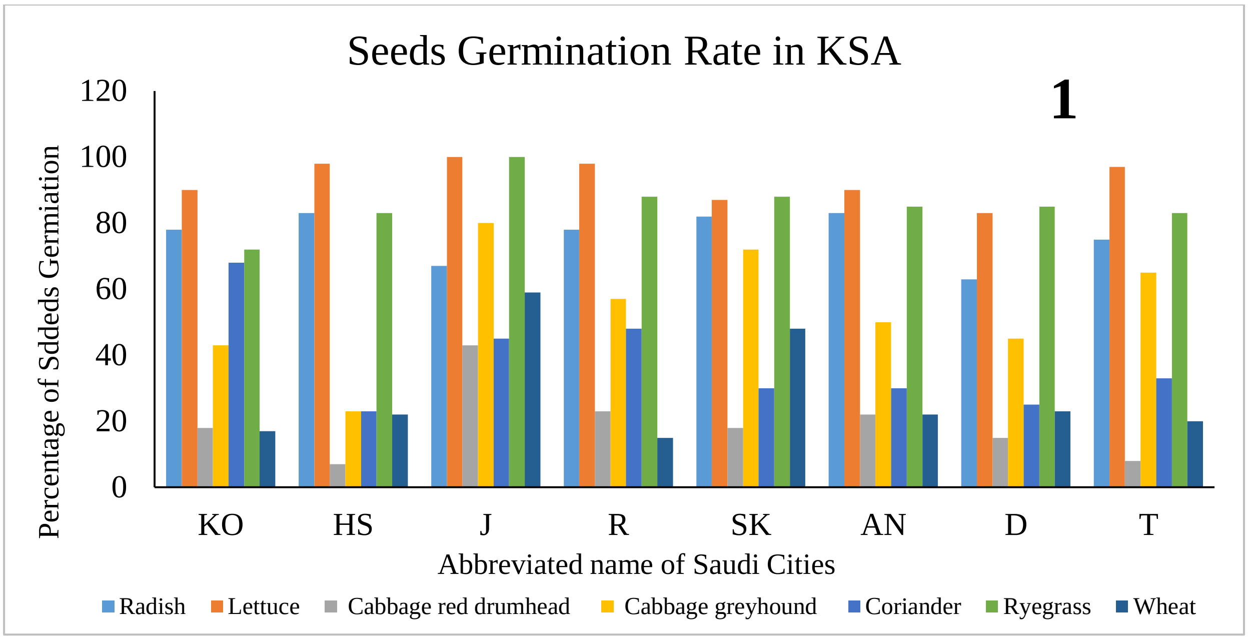


Figure 69 Comparison between Wheat root and shoot length in all Saudi sands. Bars indicate SEM of three replicates. Concentration of total petroleum hydrocarbons (red dot) of the fourteen Saudi sand samples.

3.3.15 Seeds Germination Saudi Sands Conducted in KSA.

In KSA, seven types of plants were planted in sixteen types of Saudi sands (**Figure 70**). The percentage germination varied for all samples. Cabbage Red Drumhead in sample Karj had the lowest percentage germination (5%) amongst all samples. Sample Karj also had the highest TPHs concentration (34708 mg/kg). Lettuce had the highest percentage germination in all Saudi sands followed by Ryegrass.



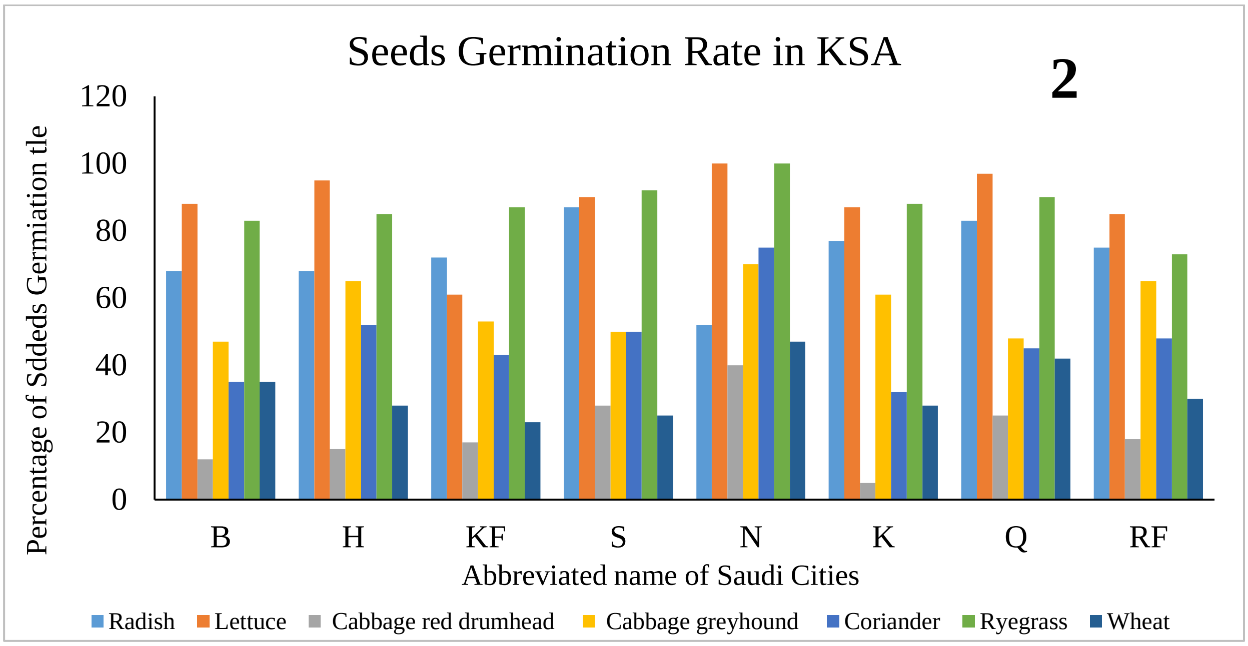


Figure 70 The percentage germination of different seeds planted into 16 different Saudi sand sampled from (1) KO, HS, J, R, SK, AN , D and T; (2) B, H, KF, S, N, K, Q, and RF. Experiments were conducted in KSA.

3.4 Discussion

The research conducted here set out to identify plant species which could be used in the phytoremediation of crude oil contaminated sand.

In 2011, the United Nations Environment Programme reported that the intervention value of total petroleum hydrocarbons (TPHs) is 5000 mg/kg (0.5%) as set by EGASPIN (Environmental Guidelines and Standard for the Petroleum Industry in Nigeria). Consequently, a sample of sand which has a concentration of TPH above 5000 mg/kg (0.5%), is considered contaminated and attention needs to be paid (UNEP).

According to a study conducted in 2011, using six plant seeds (Wheat, Maize, Cotton, tall Fescue and Mexican Corn Grass), the TPH can inhibit plant root elongation at levels above 0.5% of crude oil in sand (Tang *et al.*, 2011). It was also suggested that the critical level of TPH concentration for animal and phyto-toxicity is 1.5% and that different plant species may behave differently depending on crude oil type and sand type (Tang *et al.*, 2011).

Baruah. P *et al.* in 2016, conducted a study on the herb called *crotalaria pallida*. The aim of the study was to evaluate the potential of the plant for crude oil remediation. *Crotalaria pallida* was grown in India in contaminated sand spiked with 1% to 10% crude oil. The experiment found that the uptake of hydrocarbons by the plant increased as the concentration of crude oil in soil increased, but the shoot and root biomass reduced (Baruah, Deka and Baruah, 2016). Hydrocarbon uptake increased up to 6% crude oil, after which it decreased and at a crude oil concentration of 10% the plant could not survive.

In 2012, a study in China assessed 14 ornamental plants in the phytoremediation of petroleum contaminated sand, by determining the effect of plant growth on the degradation of TPHs and its components. The average concentration of TPHs in contaminated sand was approximately 28,000 mg/kg, consisting of aliphatic hydrocarbons (40.76%), aromatic hydrocarbons (27.02%) and asphaletenes (30.82%) (Liu *et al.*, 2012). This research reported, that the TPHs and its components could be effectively reduced to 10,000 mg/kg TPH by pot-culture of the 14 ornamental plants. The removal rate ranged from approximately 19.45% to 49.24% of TPHs in contaminated sand after remediation. The Purple Coneflower and Fawn had the largest percentage removal rates of TPHs, whereas Cornflower and Snapdragon had the lowest percentage removal of TPHs. The removal rate of most of the other plants reached more than 35% of the TPHs (Liu *et al.*, 2012). Therefore, phytoremediation using different ornamental species is considered an effective method for treatment of TPH in petroleum contaminated sand (Liu *et al.*, 2012).

In 2012, another study investigated the effect of crude oil contaminated soil on the germination rate of *Abelmoschus esculentus*. It was found that crude oil affected the growth and germination rate of *Abelmoschus esculentus*. It was found that crude oil in soil above 2% was toxic to the plant in every form (Oyedeji *et al.*, 2012).

According to Gudin and Syratt (1975), grasses can be used in soil remediation because grass has a fibrous root system with a great surface area for colonisation of microbes. These root systems can form a persistent and intense rhizosphere which leads to availability of exemplary conditions for phytoremediation.

In this research, seven types of plants were germinated and grown in a control sand and sand contaminated with crude oil from 0.5% up to 10% in both the UK and KSA. Next, germination and growth experiments were conducted in both the UK and KSA on 16 Saudi sands, containing different levels of TPHs. The concentration of TPHs recovered before and after germination was determined using both Soxhlet and 24h extraction methods.

3.4.1 Total Petroleum Hydrocarbons Yield.

Prior to germination, TPHs were extracted from crude oil contaminated sand using two different methods. Soxhlet and 24h extraction methods. Soxhlet extraction (3 Hours) recovered between 24.2% of TPHs and 60.7% of TPHs, while 24 h mechanical shaking recovered between 8.97% and 19.8% of TPHs. The highest percentage recovery was found at 1% and 0.5% of crude oil, while the lowest TPHs recovery was found at 10% crude oil for both methods.

It is also worth noting that after seed germination, TPH recovery was significantly less. Furthermore, the 24h extraction method recovered greater amounts of TPHs compared to the Soxhlet method, which suggested that most components extracted via Soxhlet extraction, were the components used for seed germination.

3.4.2 Seed Germination in control and Crude Oil Contaminated Sand, conducted in the UK and KSA.

The type of sand, climate conditions, TPHs and physio-chemical properties of the sand could play a role in the percentage germination of seeds. Factors such as seed size can also affect percentage germination. Furthermore, some seeds may germinate early while some seeds may germinate late, which then affects the time to reach the desired length before harvesting (Oyedeji *et al.,* 2012).

As expected, in this research seed germination rate also varied between the 7 seed types, which affected the growth measured at the point of harvesting. Lettuce and Ryegrass had the highest germination rate as well as the best plant growth among all plants types at crude oil levels up to 2%.

Ryegrass planted in the UK and in KSA grew well in crude oil levels up to 2% but at concentrations of 5% and 10% root and shoot length decreased compared to the control sand. There is some correlation to the results found by Baruah. P *et al.* in 2016, except at a concentration of 10% Baruah *et al*. found no survival of plants whereas, in this research the plant growth was just reduced. Therefore, Ryegrass could be grown in sand containing up to 2% crude oil for phytoremediation.

Lettuce had the same growth trend in both the UK and KSA. In both countries, there was an increase in root length at all contaminated levels, with the greatest increase seen at 2% crude oil. In both countries, shoot length for Lettuce up to 2% crude oil was unaffected, but decreased at 5% and 10% crude oil. Therefore, lettuce could also be grown in sand containing up to 2% crude oil for phytoremediation.

In the UK, Wheat had a positive response to crude oil contamination compared to the control sand. However, Wheat grown in KSA had a negative response to the contaminated sand at most concentrations. In the UK, there was an increase in root and shoot length at all levels of crude oil, except at 5% contamination. In KSA there was an increase in root and shoot length at 0.5% crude oil, but at a concentration of 2% and 5% crude oil there was an increase in shoot length only. The difference in results seen for Wheat between the UK and KSA, could be due to the high temperature in KSA, which may have evaporated most of the crude oil components.

Radish, Cabbage Red Drumhead and Cabbage Greyhound all had negative responses to contaminated sand. Interestingly, the germination rates and growth patterns seen in the UK and KSA were similar even though conditions were significantly different. However, Radish, Cabbage Red Drumhead and Cabbage Greyhound would not be recommended for phytoremediation.

Coriander had very different growth patterns in the UK and KSA for crude oil contaminated sand. In the UK, germination of Coriander was good (90% at 0.5% crude oil) but root and shoot length was less than the control sand at all crude oil levels. However, in KSA, Coriander germination was poor (13% at 0.5% crude oil) but root and shoot growth was good at all contamination levels. In KSA the temperature was higher and possibly evaporated all light hydrocarbon compounds from the contaminated sand, which resulted in better root and shoot growth in contaminated sand in KSA.

Taken together, the germination rate, root and shoot growth of different seeds varied in contaminated sand. The plants identified here which could play a role in phytoremediation of sand containing up to 2% crude oil include Ryegrass and Lettuce. Wheat may be useful in high humidity, 20˚C conditions with crude oil levels up to 10%.

3.4.3 Seed germination and growth in Saudi sands conducted in the UK and KSA.

Next, the seven types of seeds were planted in sixteen different Saudi sands and control sand. Seed germination was conducted in the UK and KSA to investigate the effect of changing climate conditions on germination and plant growth. In the UK, all conditions were completely controlled, while in KSA the conditions were not controlled.

Seed germination in Saudi sands varied among the sands as well as where the experiments were conducted. In the UK, Wheat had the lowest percentage germination in most Saudi sands (2%). In KSA, Cabbage Red Drumhead in sample Karj had the lowest percentage germination among all Saudi sands (5%). Lettuce and Ryegrass had the highest percentage germination in all Saudi sands in both the UK and KSA, which compared well with the results seen for the crude oil contaminated sand.

Sample Dammam had a TPH concentration of 3386 mg/kg, which is less than 0.5% of crude oil and thus seeds were expected to germinate well (Baruah, Deka and Baruah, 2016). In the UK, plants grew better in Dammam sand than in control sand apart from Cabbage Red

Drumhead and the roots of Cabbage Greyhound and Wheat. In KSA, Cabbage Greyhound, Coriander and the shoots of Ryegrass grew less than in control sand. It was noticed in both countries that Radish grew the most, while Cabbage Red Drumhead grew the least.

Sample Unaizah had a total TPH concentration of 2190 mg/Kg which is just above 0.2% contamination and according to UNEP in 2012, sample Unaizah is an uncontaminated sample. Both experiments had some similarity in plant growth. Wheat grew the best, while Coriander and Cabbage Red Drumhead had the least growth in the UK and KSA, respectively.

Samples Najran and Sulayyil had a TPH concentration of 28008 mg/kg and 20347 mg/kg respectively. Both samples contained less than 3% crude oil, but according to UNEP in 2012, these samples require treatment. Results in the UK and KSA were almost identical in terms of root and shoot growth. In sample Sulayyil, Cabbage Red Drumhead, Coriander growth was more than in the control sand. The roots of Lettuce and Wheat also grew better than in control sand. In sample Najran, Lettuce, Cabbage Red Drumhead, Coriander, Wheat and Radish grew better than in control sand. These results differ from the results seen in crude oil spiked samples. This could be due to the Saudi sands having different characteristics.

Samples Riyadh and Jeddah had a TPH concentration of 1414 mg/kg and 618 mg/kg, respectively, which is below 0.2% of crude oil and good germination and growth of seeds was expected. The growth patterns seen in the UK and KSA were quite similar.

Samples Buraidah, Hufuf, Karj, Khafji, Skaka, Al-Hassy, Khobar, Qatif, Tabuk and Rafha had TPHs concentrations which ranged from 236 mg/kg up to 34708 mg/kg. There was a large variation in results between UK and KSA experiments, which was most likely due to the difference in temperature and amount of water given between the UK and KSA. Plants in KSA received three times more water than in the UK study.

The differences in plants growth between each sample and between the two countries could be linked to the type of sand, different conditions of the experiments such as temperature and humidity, and the amount of water plants received.

4 Chapter 4: Remediation of Hydrocarbon Contaminated Sand with Surfactants.

4.1 Introduction.

Oil spills contaminate natural environments with both aliphatic and aromatic compounds. Environmental remediation technologies for hydrocarbon reduction could be chemical, physical or biological techniques (Agarwal and Liu, 2015). Ex-situ soil washing is considered to be one of the effective technologies in order to clean up hydrocarbons contamination and reclaim the natural sand, sediment or soil. The hydrocarbon contamination components cannot be removed by only water, therefore, additives such as surfactants are required in order to increase solubility (Agarwal and Liu, 2015) (Mao *et al.*, 2015). Hydrophilic and hydrophobic moieties are able to change the medium properties. In addition, surfactants are able to form aggregates in the water medium (CMC) (Paria, 2008). However, some of the agents could pose acute environmental problems, because of their resistance to biodegradation and toxicity (either direct or indirect) (Ackson *et al.*, 2016). Therefore, a further treatment process for the reclaimed sand and water used in the washing process would be required in order to remove both contaminants and surfactants (Trellu *et al.*, 2016).

Soil, in particular sandy soil, can be described as a complex porous and solid matrix, thus making the treatment of contamination more laborious. The quality of soil is largely affected by the presence of hydrophobic contaminants such as petroleum hydrocarbons, polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) from oil industries or processing. These organic contaminants are generally characterised by having a low aqueous solubility and high interfacial tensions with water, so they may leach from the soil for a longer period of time.

The presence of contaminants such as polycyclic aromatic hydrocarbons and petroleum hydrocarbons in long-term contamination of soils, constitutes a common concern as these compounds have high potential toxicological impact. Lately, all processes used for soil remediation have at least one important disadvantage such as high costs, high perturbation of the soil texture, low efficiency, long treatment time requirements (biodegradation processes). (Trellu *et al*., 2016). Therefore, the need to use a cost-effective process with high pollutant removal efficiency is required, however, this has become a major challenge for researchers and soil remediation specialist. In situ-scale applications, of some treatment methods (such as bioremediation, incineration and phytoremediation) which destroy the contaminants, are time consuming and not cost effective when handling large quantities of the contaminated material, while others separate the contaminants from soil without chemically modifying them (e.g. Soil washing) (Trellu *et al.*, 2016).

For the past 20 years, soil washing processes using extracting agents (surfactants and bio surfactants) have been reported to show very promising results (Mulligan *et al*., 2001). The soil washing processes enhanced by the use of extracting agents (surfactants, bio surfactants, etc.) can be describe as a conceivable, cost effective, relatively fast and efficient approach has the potential to treat and recover large volumes of contaminants The approach of soil washing also prevents the generation of harmful by-products that may be produced during remediation processes (Trellu *et al.*, 2016). This treatment method has a good potential, particularly for the treatment of polluted soils most especially in the case of Saudi Arabia which is predominately sand, where an environmentally friendly and efficient remediation technology is a major challenge.

Soil washing uses liquids (usually water, occasionally combined with certain solvents) and mechanical shaking processes to scrub, soils. The extracting solvents used are usually chosen on the basis of their ability to solubilize specific contaminants, and on their environmental and health effects (Feng *et al*., 2001; Chu and Chan, 2003; Urum *et al.*, 2003). Soil washing process can be described as an *ex situ* process, i.e., the soil has to be sampled or excavated before the treatment. The process works based on certain solid/liquid ratio, in the range of 1-100% (Mousset *et al*., 2014) and most usually between 5 - 40%. Extracting agents are normally applied to the system in order to improve the solubility, desorption or removal of contaminants sorbed to soil and biodegradation of soil pollutants, since it is the main controlling removal mechanism (Mulligan *et al.*, 2001; Yang *et al.*, 2006). The soil washing process increases the interaction between extracting agents and soil pollutants, thereby allowing better treatment efficiency and contact time. In general, extracting agents helps to enhance the availability or aqueous solubility of soil contaminants (i.e. Petroleum hydrocarbons) for the reactive oxygen species by relocating them into the aqueous phase. These extracting agents can also reduce the time necessary to treat a site compared to the use of water alone. Aside from their extracting and solubilizing abilities, they must be of low ecotoxicity for the soil and biodegradable (Mulligan et al., 2001). Example of commonly used extracting agents apart from water are surfactants

The term surface-active agent or “surfactant” represents a diverse group of inexpensive important chemical compounds widely used in manufacturing cleaning detergents, personal care products, and can be used in various industrial applications (e.g., oil, textiles, polymers, agriculture, paints). Surfactants are comprised of a heterogeneous long-chain molecule containing both hydrophilic (head) and hydrophobic (tail) moieties and are grouped by their ionic properties in water as either anionic (negative charge), non-ionic (no charge), cationic (positive charge), or amphoteric (positive/negative charge depending on pH).

When considering the choice of surfactant for remediation (e.g. soil washing), both the surfactant's biodegradability and its toxicity are important factors. Biodegradation is defined as a process by which living organisms transform, remove or alter the structure of chemical compounds through metabolic or enzymatic activities. Toxicity, on the other hand, describes the degree of harmfulness of a compound. Toxicity can be classified as acute or chronic and is generally expressed as an LD_{50} (dosage) or LC_{50} (concentration) required to kill 50% of a target population.

The non-polar chain (hydrophobic) of these surfactants are either alkane, alcohols, alkyl-benzene, or alkyl-phenols. The anionic, hydrophilic group, (polar, water-loving) is either a sulphonate, sulphate, or carboxylate group. The cationic, hydrophilic group (polar) is usually a quaternary ammonium group. Polyoxyethylenes, sucrose, and polypeptides are polar groups in non-ionic surfactants (Urum, 2004).

The effects of surfactants on the biodegradation and washing of crude oil contaminated soils have been reported (Villa *et al*., 2010). In Mao *et al.*, (2014), sodium dodecyl sulfate (SDS) among other commercial surfactants was evaluated for treating petroleum hydrocarbon contaminated soil through soil washing. In the study, the authors used a surfactant solution to transfer a matrix of crude oil contaminants to a liquid phase, the surfactant solutions were then used to enhance the biodegradation of the oil. The study concluded that the application of surfactants in soil washing depends on the physiochemical nature of the surfactants and the contaminated soil.

Among extracting agents that can be used for soil washing, synthetic surfactants have been reported to have the good extraction efficiency. However, some of them have low biodegradability and are affected by precipitation or sorption onto soil, requiring larger quantities and causing possible damages to soil structure. There are four major groups of surfactant categories, examples of include anionic surfactant include; sodium dodecyl sulfate (SDS) or linear alkyl benzene sulfonate (LAS), cationic such as quaternary derivatives, amphoteric such as acocoamidopropyl hydroxide (CAS) and non-ionic such as Brij 35(B35), Tween 80 (TW80). The non-ionic surfactant is usually preferred because their lower soil sorption ability, higher solubilization capacity and higher cost-effectiveness (Trellu *et al.*, 2016).

This chapter would evaluate effectiveness of a synthetic surfactant, sodium dodecyl sulphate (SDS) and consumer goods (washing up liquid, shower gel and shampoos) in the removal of crude oil from contaminated control sand samples using the soil washing process. The soil washing parameters and ranges tested that were tested includes; shaking time and surfactant concentration. The contaminated soils were prepared in the laboratory by mixing crude oil and sands using an organic solvent. The efficiency of crude oil removal process by the different SDS and consumer goods was also further investigated.

4.2 Materials and Methods

4.2.1 Materials.

All chemicals were of reagent grade and have been used without further purification. Analytical grade hexane and dichloromethane (DCM) were purchased from Fisher scientific. Crude Oil was obtained from the UK. Sodium sulfate anhydrous and silica gel were obtained from Fisher Scientific. Saudi Sand samples have been collected from sixteen different cities in the Kingdom of Saudi Arabia (**Figure 10** (**page No 30**)). The control sand (CS) was purchased from a shop in Bangor Gwynedd (Treborth Garden Centre). SDS was purchased from Fisher Scientific. Shampoo and shower gel were purchased from Super Drugs shop in Bangor (Gwynedd). Washing up liquid was purchased from ASDA store in Bangor (Gwynedd).

4.2.2 SDS Preparation.

In order to achieve a 0.1% (w/v) of SDS, 1 g of SDS was weighed and placed into a 1000 ml volumetric flask. Deionized water was added to reach a final volume of 1000 ml. The solution was shaken for a couple of minutes to dissolve the SDS in deionized water.

4.2.3 Consumer Goods Preparation.

Three types of consumer goods were purchased (shampoo, shower gel and washing up liquid) from a shop (Superdrug) in Bangor, North Wales (**Figure 71**). In order to prepare an approximately 0.1% surfactant solution of the consumer goods, 5 ml (one tea spoon) was added into a 1000 ml volumetric flask. Deionized water was added to reach a final volume of 1000 ml. The solution was shaken for a couple of minutes to mix the two liquids in deionized water.

Figure 71 The consumer goods used in the experiment: shower gel (A), shampoo (B) and washing up liquid (C).

4.2.4 Sand Contamination with Crude Oil.

Horticultural sand (100 g) was contaminated with 2 g, 5 g and 10 g of crude oil in a conical flask to reach 2%, 5% and 10% of crude oil contamination (w/w), respectively. Each 1 g of crude oil equivalent to 1.36 ml of crude oil. A desired volume of a mixture of DCM-Hexane (1:1) was added on top of crude oil. The mixture was shaken using a mechanical shaker for 2 to 3 min in order to solubilize the crude oil. Then 100 g of sand was added gradually into the conical flask to be contaminated. The mixture was shaken for a further 30 minutes and left to sit overnight to allow evaporation. Afterwards, the samples were shaken again for 3 minutes. To assure complete removal of the hexane and DCM, the samples needed to be left uncovered for at least 24 h (Tang *et al.,* 2011) (Baruah, Deka and Baruah, 2016) .

4.2.5 TPHs Recovery from Contaminated Sand Using SDS and consumer goods Solution.

The test for sand samples was done by taking five parts SDS solution and one part sand (ratio 1:5 (w:v)) in a glass vial. 5 g of contaminated sand was added into a 30 ml glass vial. 25 ml of 0.1 % SDS added into the glass vial along with a stirrer bar. The vial was left stirring on a heating mantle for either 20, 40 or 60 minutes. Then the samples were left to stand overnight in order to settle. The next day, the solution was transferred into a clean 30 ml glass vial, and the original sample vial was rinsed with 10 ml of deionized water and the two fractions combined. Then 20 ml of DCM was added into the separating funnel, followed by the SDS solution. 30 ml of saturated brine solution was also added into the mixture in the separating funnel in order to break the foam down. The funnel was sealed and shaken for two to three minutes, three times. Between each shaking, the funnel needs a periodic venting in order to release excess pressure. Then leave the organic extraction for three to five minutes in order to separate from the water phase. Then collect the solvent extract in a beaker. Repeat the extraction two more time each time with 20 ml of DCM and follow the same procedure. Then combine the three fractions together in a beaker. Add sufficient amount of anhydrous sodium sulfate in the fractions in the beaker in order to repel any water in the fractions. Use filter paper to filter the fractions in round bottom flask. Evaporate the fraction using rotary evaporator until dryness. Dissolve the residue in 1 ml of hexane. Transfer the solvent into GC vial and send it to GC-MS for analysis.

4.2.6 Total Petroleum Hydrocarbon Yield from SDS and Consumer Goods.

The percentage of total petroleum hydrocarbon were determined using the same recovery method described above (**4.2.4**). The solution was collected into pre-weighed glass vials and evaporated until dry using rotary evaporation. The glass vials containing the residue were then re-weighed to calculate the percentage of total petroleum hydrocarbon from the contaminated sand solution.

TPHs Yield = weight of round bottom flask after evaporation – weight of empty round bottom flask

4.3 Results

SDS, shampoo, washing up liquid and shower gel recovered total petroleum hydrocarbons from contaminated sand. The recovered percentage varied depend on type of surfactants, concentration of surfactant solution, solution shaking speed and concentration of sand contamination. Solutions in experiment were transferred into glass vials and conical flaks after shaking. Samples were left to settle until the sand went to the bottom of glasses (**Figure 72**).

Figure 72 Image of sodium dodecyl sulfate (SDS) and consumer goods (shampoo, shower gel and washing up liquid) solution from contaminated sand during the TPHs recovery experiments.

In this experiment, SDS and consumer goods solution formed a big layer of foam on top of the solution after shaking together in a separating funnel. This layer hindered getting the solvent, which extracted hydrocarbons from contaminated sand. Therefore, 30 ml of saturated brine was added into the separating funnel in order to break the foam down (**Figure 73**).

Consequently, after adding the saturated brine solution was it simple to get the solvent, which extracted hydrocarbons.

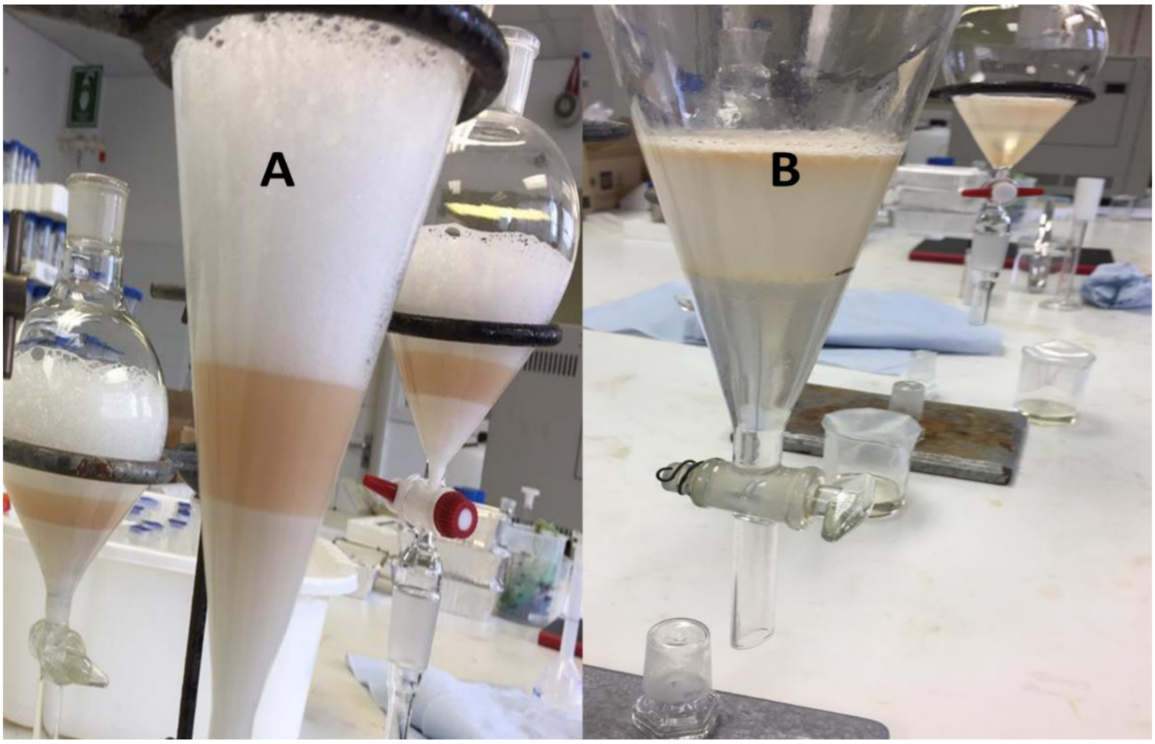


Figure 73 Image of sodium dodecyl sulfate (SDS) and goods (shampoo, shower gel and washing up liquid) during the TPHs separation processes, showing the solution before (A) and after (B) addition of saturated brine.

4.3.1 Total Petroleum Hydrocarbons recovered by 0.1 % of SDS

The following figure shows that, SDS (0.1% w/v) solution recovered TPHs from contaminated sand (**Figure 74**). There was a clear increase in TPHs recovery by increasing crude oil concentration. At 2% of crude oil contamination, the recovered TPH was 9%. At 5% of crude oil contamination, the recovered TPH was 10%. However, at 10% of crude oil contamination, the recovered TPH was 24%. Therefore, SDS was more efficient at recovering TPHs at 10% crude oil contamination, rather than lower contamination concentrations.

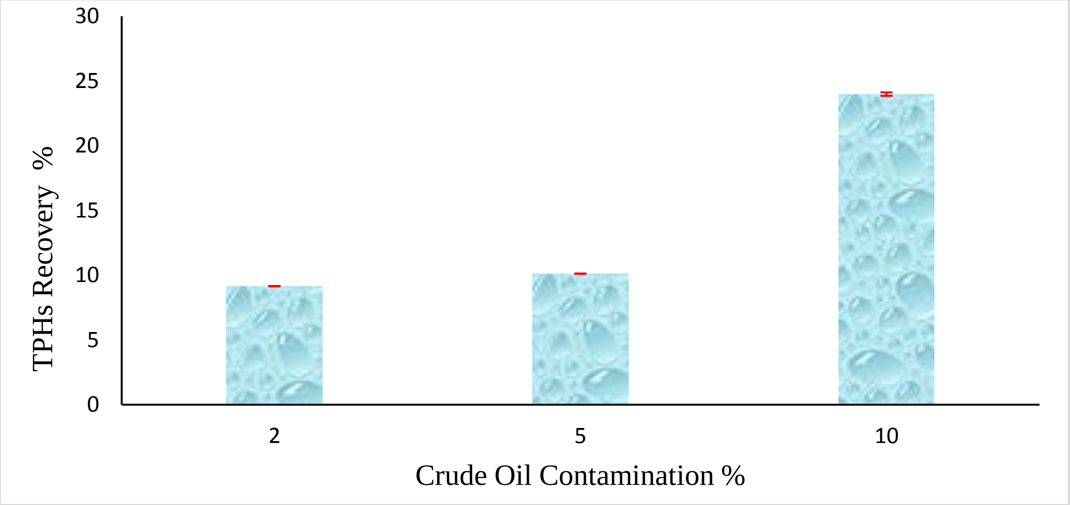


Figure 74 Percentage of TPHs recovery using SDS 0.1% (w/v) for 20 minutes, stirring at 350 rpm. Bars indicate SEM of three replicates.

In the following figure, there was an obvious increase in recovered TPHs from contaminated sand (**Figure 75**). At 2% of crude oil contamination, the recovery percentage was 9.5% of TPHs. At 5% of crude oil contamination, SDS recovered 15.5% of TPHs. At 10% of crude oil contamination, SDS recovered 20.5% of TPHs. By increasing the crude oil contamination level, the more TPHs were extracted using SDS.

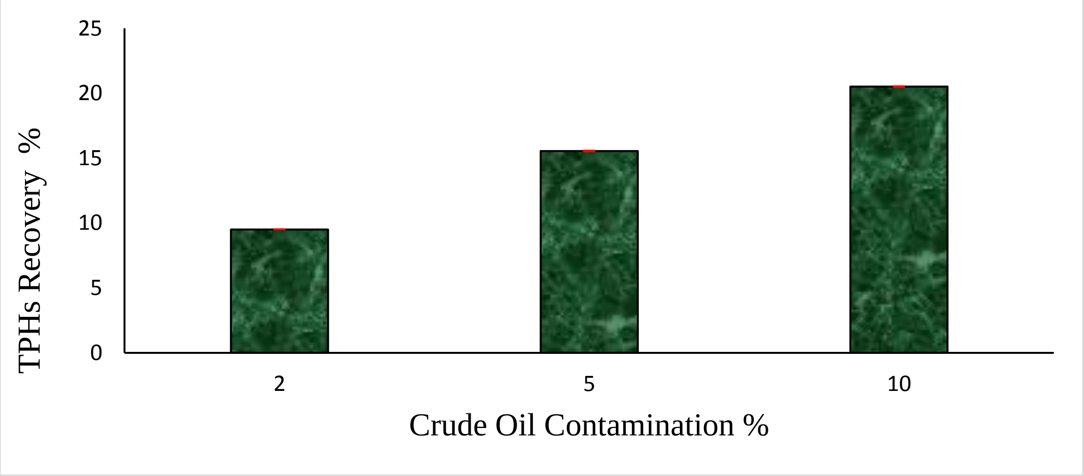


Figure 75 Percentage of TPHs recovery using SDS 0.1% for 40 minutes shaking at 350 rpm. Bars indicate SEM of three replicates.

Figure 76 shows a gradual increase in recovered TPHs using 0.1% of SDS. At 2% of crude oil contamination, SDS recovered 10.6% of TPHs. At 5% of crude oil contamination, SDS recovered 18% of TPHs, while at 10% of contamination; the recovery percentage was 22% of TPHs.

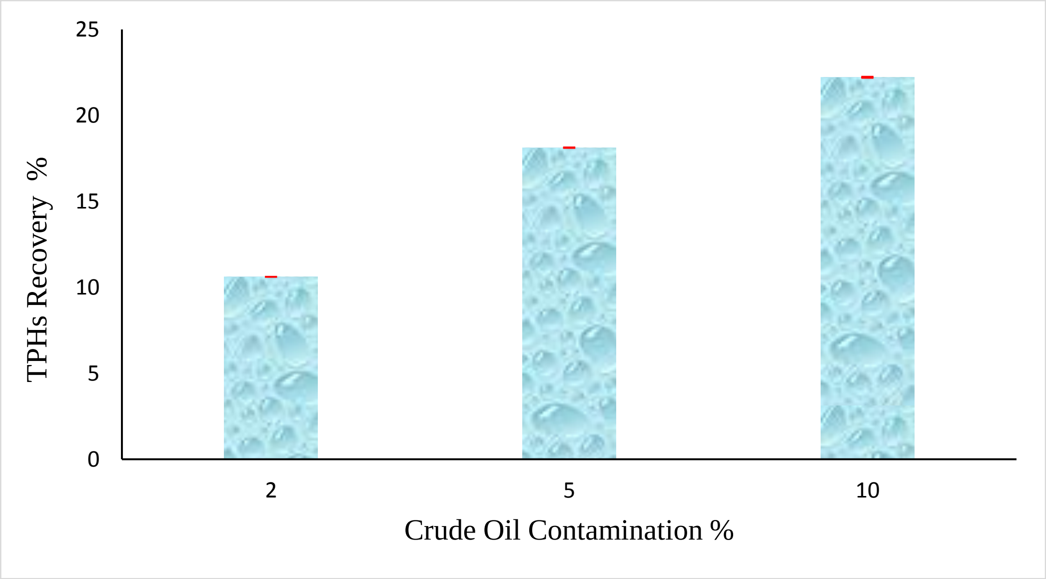


Figure 76 Percentage of TPHs recovery using SDS 0.1% (w/v) for 60 minutes stirring at 350 rpm. Bars indicate SEM of three replicates.

4.3.2 Total Petroleum Hydrocarbon Recovered using Consumer Goods

Based on the initial trials using SDS only, the optimised parameters of 60 minutes shaking and 0.1% (w/v) SDS at 350 rpm were used, but the SDS solution was replaced with consumer goods. This was done to illustrate the potential for simple remediation using resources locally available such as washing up liquid, shower gel and shampoo. It was assumed that the SDS concentration in these consumer good was around 25%. **Figure 77** shows that shampoo (0.1%) at 60 minutes had clear increase in TPHs recovery by increasing the crude oil contamination levels. At 2% of crude oil contamination, the TPHs recovery found to be 5% of TPHs, and then increased to 11.5% at 5% of contamination. At 10% of contamination, the recovered TPHs was 17%.

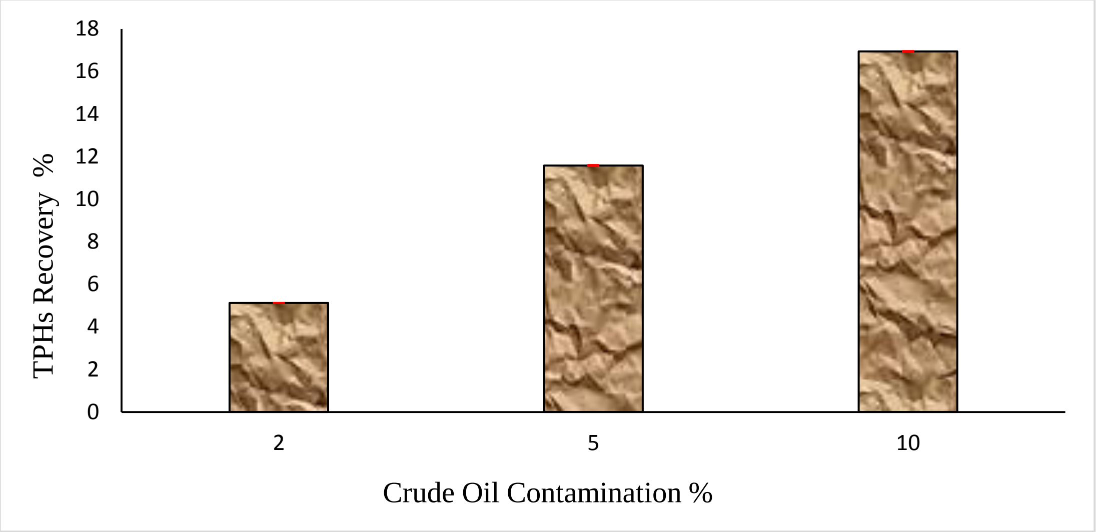


Figure 77 Percentage of TPHs recovery using shampoo 0.1% (w/v) for 60 minutes stirring at 350 rpm. Bars Indicate SEM of three replicates.

The following figure shows that 0.1% of shower gel had a gradual increase in the recovered TPHs by increasing the crude oil contamination levels (**Figure 78**). At 2% of crude oil contamination, the TPHs recovery found to be 12% of TPHs, and then increased to 17% at 5% of contamination. At 10% of contamination, the recovered TPHs was 18%.

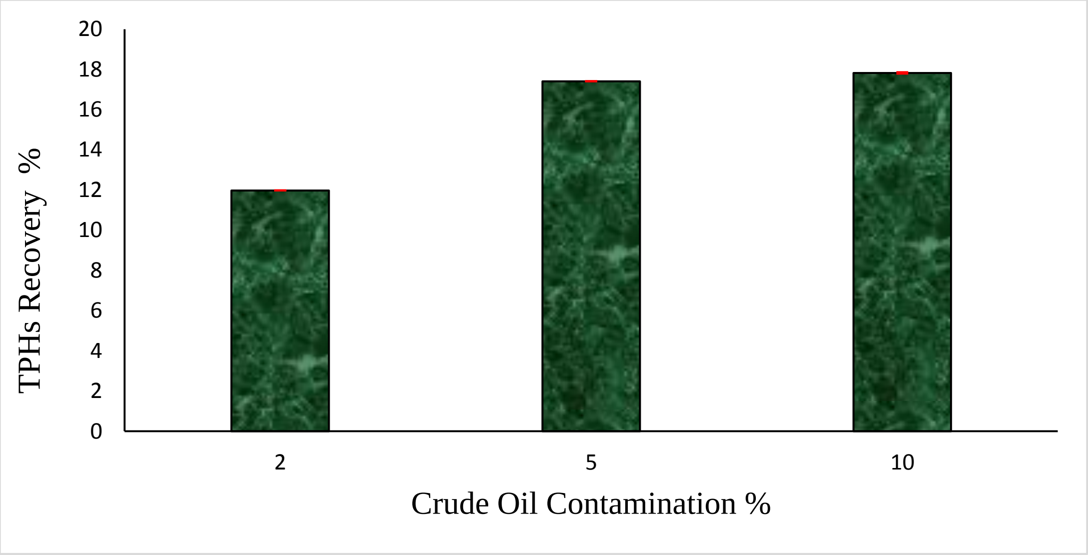


Figure 78 Percentage of TPHs recovery using shower gel 0.1% (w/v) for 60 minutes stirring at 350 rpm. Bars Indicate SEM of three replicates.

Figure 79 shows that washing up liquid (0.1%) at 60 minutes had a clear increase in TPHs recovery by increasing the crude oil contamination levels. At 2% of crude oil contamination, the TPHs recovery found to be 15% of TPHs, and then increased to 17% at 5% of contamination. At 10% of contamination, the recovered TPHs was 20.5%.

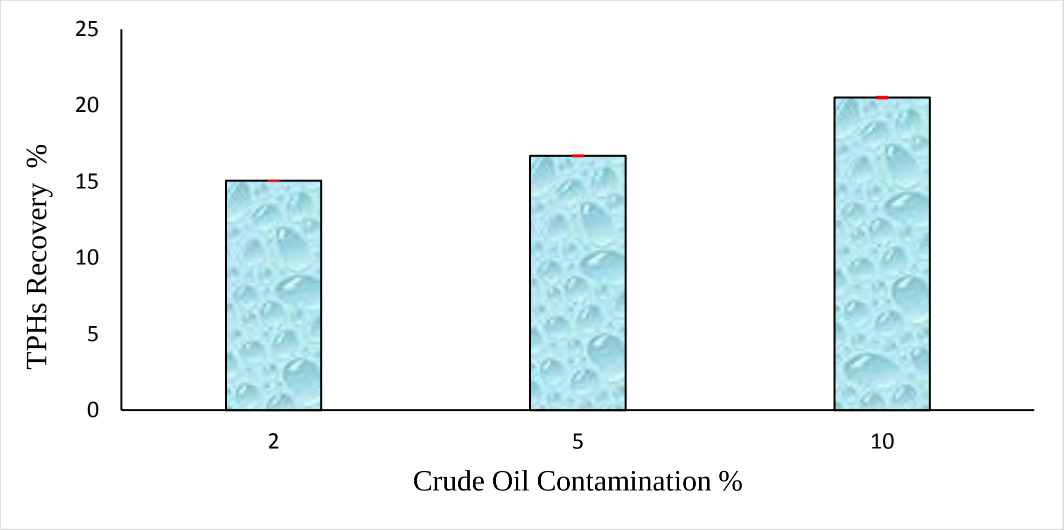


Figure 79 Percentage of TPHs recovery using washing up liquid 0.1% (w/v) for 60 minutes stirring at 350 rpm. Bars Indicate SEM of three replicates.

4.4 Discussion

Kingsley Urum *et al*. (2006) had conducted a study to investigate the efficiency of different types of surfactants to remove crude oil from polluted soil. The study used SDS, rhamnolipid and saponin. The outcome of the study was found that SDS removed the most crude oil from polluted soil (46% of crude oil removed). However, saponin removed the least crude oil from polluted soil (27% of crude oil removed). In comparison, rhamnolipid removed 44% of crude oil from contaminated soil. In addition, it was found that SDS removed more of the aliphatic hydrocarbon fraction than aromatic fraction. Whereas, saponin removed more of the aromatic hydrocarbon fraction than aliphatic fraction. The reason for low removal percentage of crude oil from polluted soil could be the effects of weathering polluted soil. Beside the removal of volatile compounds of weathering soil and difficulty of remove high molecular weight such as aromatic, asphaletene and non-organics (Urum *et al.*, 2006).

In 2004, Kingsley Urum *et al.,* conducted a study on the recovery of crude oil from contaminated soil (four types of soil) using synthetic (SDS) and biological (rhamnolipids) surfactants. Kingsley Urum *et al.* conducted their study on weathered and non-weathered crude oil. Therefore, the non-weathered crude oil, at concentrations of 0.004% to 0.5% of SDS solution, recovered crude oil from 40% to 90%. Whereas, at the same concentration, rhamnolipid removed crude oil from 38% to 85%. In addition, at 5 ml to 20 ml volume of SDS solution, the removed crude oil ranged from 45% to 90%. Whereas, at the same volume, the rhamnolipid removal ranged from 50% to 80% of crude oil. Moreover, at a speed of 80 rpm to 200 rpm, the SDS removal ranged from 25% to 90% of crude oil, while rhamnolipid at the same shaking speed removed from 10% to 80% of the contamination. Regarding the washing time, the time ranged from 5 minutes to 20 minutes, and SDS removed from 55% to 90% of crude oil at 25 minutes; rhamnolipid removed from 50% to 85% of the contamination at the same washing time (Urum, Pekdemir *et al.,* 2004).

However, the weathered crude oil, at a concentration of 0.004% to 0.5% of SDS solution, recovered crude oil from 5% to 70%, respectively. Whereas, at the same concentration, rhamnolipid removed crude oil from 5% to 55%, respectively. In addition, at 5 ml to 20 ml volume of SDS solution, the removed crude oil ranged from 2% to 65%, respectively. Whereas, at the same volume, the rhamnolipid removal ranged from 2% to 60%, respectively of crude oil. Moreover, at a speed of 80 rpm to 200 rpm, the SDS removal ranged from 0% to

65% of crude oil. While rhamnolipid at the same speed shaking removed from 0% to 50% of the contamination. Regarding the washing time, the time ranged from 5 minutes to 20 minutes, and SDS removed from 0% to 60% of crude oil at 25 minutes. While rhamnolipid removed from 2% to 50% of the contamination at the same washing time (Urum, Pekdemir *et al.*, 2004).

Harvey *et al.* in 1990 reported that some bio-surfactants produced from *Pseudomonas aeruginosa*, were applied in an Alaskan beach contamination site. The study compared two different extraction methods. The study used water at a high temperature and 0.1% of biological surfactant to remove crude oil contamination from sand. The outcome was that the biological surfactant extracted three times better than using water at a high temperature especially at 30 ^{o}C and above compared to a room temperature (Harvey *et al.*, 1990). The study used different parameters such as temperature range (10 ^{o}C to 80 ^{o}C), contact time (0.5 minutes to 2 minutes) and range of surfactant concentration (0.001% to 1%). It was found that by increasing the temperature, the oil removal increased at 30 ^{o}C, the surfactant (0.1%) removed approximately 15% of the contamination. While at 50 ^{o}C, the surfactant (0.1%) removed 37% of the contamination. In addition, at 1% of surfactant, the removal of contamination was 70% at 80 ^{o}C (Harvey *et al.*, 1990).

In this project, it was observed that there was an increase in TPHs recovery using SDS by increasing the crude oil contamination and increasing the shaking time (**Figure 80**). Therefore, at 60 minutes shaking time, the TPHs recovery was higher than at 20 and 40 minutes at all contamination levels. The highest shaking time and the highest contamination level, the maximum TPHs recovered. By comparing these results to the study by Kingsley Urum *et al.*, (2006), it was found that in this project, SDS extracted less than that reported in the study. In this project, the parameters were 60 minutes time shaking and 30 ml of 0.1% SDS solution. However, the SDS used in the study removed 46% of crude oil contamination, while in this project; the removal of crude oil was 22%; this removal was less than half of the reported study (Urum *et al.*, 2006). This difference could refer to the difference in crude oil type, parameter conditions or sand type. In this project the temperature was set as room temperature while the reported study conducted at 20 ^{o}C which could be a room temperature. In addition, the crude oil used in the reported study was weathered at 50 ^{o}C for 14 days in an oven. This could affect the removal of crude oil process. However, in this project the crude oil was not weathered and used at room temperature. In the study by Kingsley Urum *et al.*, (2006), saponin removed 27% of crude oil from the contaminated soil, while rhamnolipid removed 44% of crude oil. By comparing the removal of both saponin and rhamnolipid to the removal of SDS in this project, it was found that saponin was similar to SDS removal, while rhamnolipid removed twice as much oil (44%) as SDS removal (22%) of crude oil in this project.

From the results of the three different contamination leveles at a differenet shaking time **Figure 80,** the results showed that at all shaking time, the TPHs recovery was higher at 10 % of contamination followed by 5 % and 2 % of crude oil contamination. The result (**Figure 80**) also showed that there was no significant difference between each concentration (2 % to 10 %) at all shaking time apart from at 5 % of contamination ($p < 005$); there was a significant difference between 5 % at 20 and 60 minutes as the *p*-value was 0.0103. In addition, *p* value of the three contmniantion levels (2, 5 and 10 %) at each shaking time (20, 40 and 60 minutes) was analysed. There was highly sigificant difference between each concentration **(Appendix 13)**. At 60 minutes shaking, there was a highly significant difference between 2 % and 10 % as the *p* value was 0.0006. however, at 20 and 40 minutes shaking time, there was no significant difference between 2 % and 10 % as the $p < 0.05$.

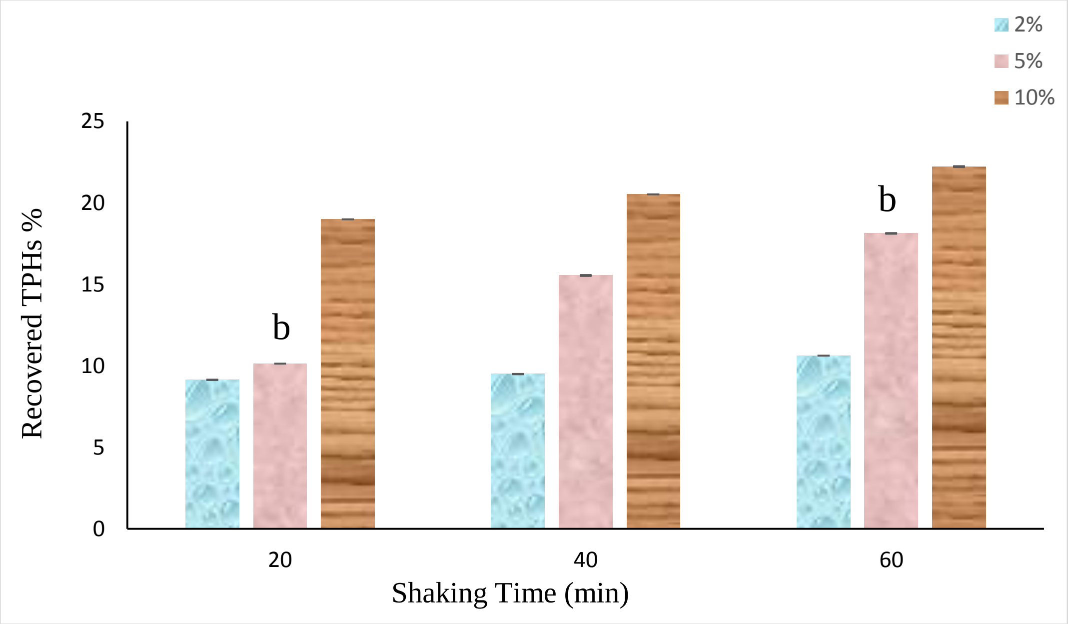


Figure 80 Total petroleum hydrocarbons recovery from three different crude oil contamination levels of control sand at three different shaking time using SDS solution. Bars indicate SEM of three replicates. Lettering a, b and c refers to the results of t-tests undertaken

where a significant difference ($p < 0.05$) is indicated with “a” when compared to the 2 %, “b” when compared to 5 % and “c” when compared to 10 % of crude oil contamination among all the three shaking time.

By increasing the concentration of surfactants, the total removal of crude oil from contaminated sand increased. Increasing the temperature between 20 to 50 ^{o}C, would increase the removal of hydrocarbons from contaminated sand. However, most of crude oil (weathered) can be removed in temperatures above 50 ^{o}C. An increase in the volume of surfactant solution led to an increase in the total removal of crude oil using surfactants. An increase in shaking speed from 80 to 200 strokes/min, increased the removal of crude oil in contaminated sand (Kingsley Urum *et al.* 2004).

In this project, it was found that TPHs was recovered more efficiently at 10% of contamination (60 minutes at 350 rpm, 22% recovery). However, this recovery percentage was lower than the recovery percentage in the Urum’s study. It was found that the recovery percentage of the non-weathered crude oil was higher than the recovery of crude oil in this project. This could be because of the temperature factor as in the reported study; temperatures were used over a wide range - from 5 ^{o}C to 50 ^{o}C. In addition, increasing temperature would lead to a decrease in crude oil viscosity, which would then increase the crude oil mobility and interaction with surfactant solution. Therefore, increasing temperature and surfactants concentration could play an important role in increasing the removed crude oil (Kingsley Urum *et al.* 2004).

However, the study conducted by Urum *et al.* (2004), was also conducted on weathered crude oil. By comparing the results with the reported study, there were some similarities. For example, at a concentration of 0.1% of SDS solution, this study removed 22% of crude oil compared with a crude oil removal ranging from 10% to 65% (depending on the type of soil). Therefore, this would confirm that the type of soil could play a crucial role in crude oil recovery using SDS solution. In addition, in this study, the SDS solution volume was 30 ml, and removed 22% of contamination, whilst in the reported study, using 20 ml of SDS solution removed oil ranging from 20% to 50% (depending on the type of soil). Coincidently, the weathered crude oil had some similarities with the results in this project. This could be because the weathered crude oil had volatilised low and medium molecular weight compounds. Besides that, high molecular weight compounds could not be easily removed using SDS solution.

Therefore, from **Figure 80**, it is clear that by increasing the shaking time, the higher TPHs. This resulted in 60 minutes shaking time being applied to the rest of experiments using customer goods such as shampoo, shower gel and washing up liquid. In addition, this project could state that hydrocarbons need more time in order to be removed from sand particles as the high molecular weight compounds required more time to be removed.

Regarding the consumer goods in the removal of crude oil contamination, it has been noticed that the recovered TPHs levels increased by increasing the contamination levels of crude oil for all the consumer goods (**Figure 81**). In addition, the washing up liquid was found to have the highest TPHs recovery at all levels of crude oil contamination. However, shampoo was found to have the lowest TPHs recovery. It was assumed that washing up liquid has more SDS components than shampoo and shower gel because washing up liquid is usually used for washing dishes, which have more oil and need an effective washing up liquid to remove the oil.

From the results of the three different contamination leveles using a differenet consumer goods **Figure 81,** the results showed that the TPHs recovery was higher using washing up liquid followed by shower gel and shampoo. The result (**Figure 81**) also showed that there was no significant difference between the three consumer goods at 10 % of crude oil contamination ($p < 005$). While there was a highly significant difference between shampoo and shower gel at 5 % of contamination because the p value was 0.0066. In addition, there was a highly significant difference between shampoo and showr gel and between shampoo and washing up liqud at 2 % of the contamination as the p value was 0.0049 and 0.000079, respictively. In addition at each crude oil contamination levels (2, 5 and 10 %), p value of different consumer goods (shampoo, shower gel and washing up liquid) was analysed. There was highly sigificant and sginificant difference between each consumer goods at each concentration **(Appendix 14)**. Among the consumer goods, there was a highly significant difference between 2 % and 5 % of contamination using shampoo as the p value 0.003. In addition, the was a highly significant difference between 5 % and 10 % of contamination using shampoo as the p value 0.0006.

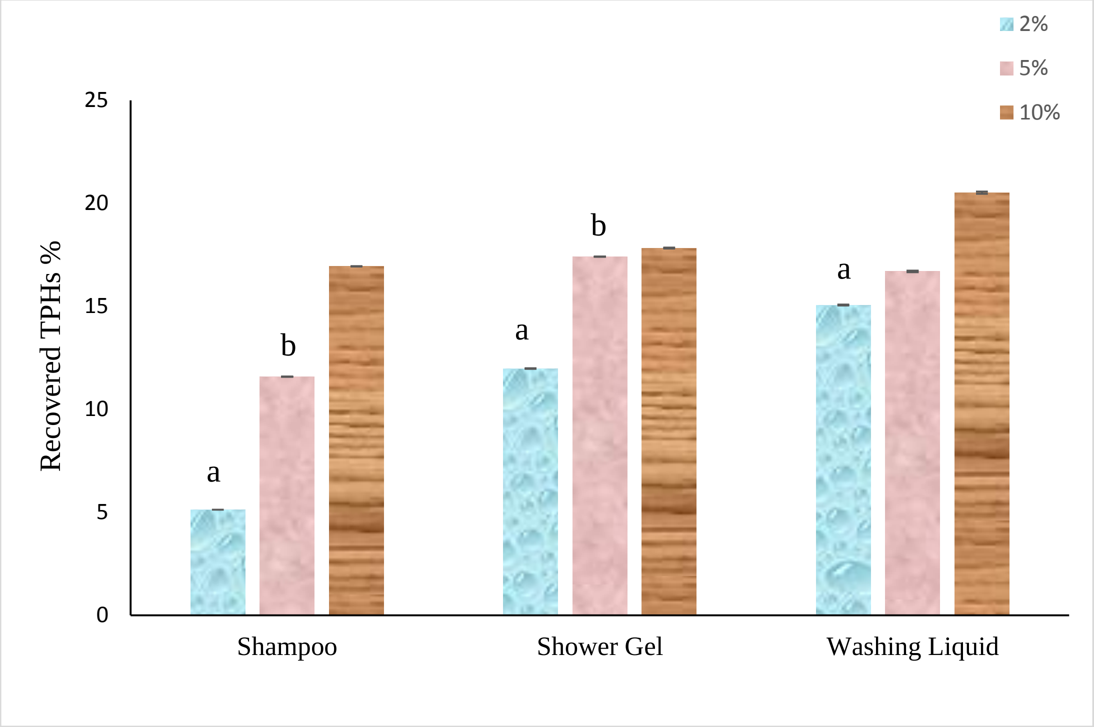


Figure 81 Total petroleum hydrocarbons recovery at three different contamination levels of crude oil using three different consumer goods. Bars Indicate SEM of three replicates. Lettering a, b and c refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with "a" when compared to the 2 %, "b" when compared to 5 % and "c" when compared to 10 % of crude oil contamination within each consumer goods.

There was an increase in the recovery of TPHs from the contaminated sand at all concentration levels. By comparing these results to the previous figure (**Figure 81**), there were some similarities. However, SDS solution (0.1%) removed crude oil contamination slightly more efficiently than washing up liquid. At 2% of the contamination, SDS removed less (11%) of the contamination than washing up liquid (15%). In addition, at 5% of the contamination, SDS removed more (18.2%) of the contamination than washing up liquid (16.7%). Moreover, at 10% of the contamination SDS removed more (22%) of the contamination than washing up liquid (20.5%). From this it can be concluded that SDS solution (0.1%) is more effective for crude oil removal from sand. However, both SDS and the consumer goods had some similarities in removing the oil contamination.

By comparing these results to the reported studies by Kingsley Urum *et al.* (2004 and 2006) and Harvey *et al.* (1990), the removal of contamination were lower than the reported studies.

Some factors to be taken into consideration such as the type of sand or soil, type of oil contamination, type of surfactants, range of temperature, shaking speeds, contact time and surfactants concentration. From the reported studies, it was found that by increasing most of the stated factors, more TPHs were recovered from the contaminated sand. As this project did not use a variation in temperature and surfactant concentration, this could be one reason for removing less oil than the reported studies. Increasing the temperature would lead to the increase of oil viscosity, which would increase the mobility of crude oil and interaction of surfactants (Urum, Pekdemir and Çopur, 2004).

Missing, low removal for consumer goods, maybe suggest to repeat washing multiple times to increase removal.

Washing up liquid is an effective detergent at remediation of hydrocarbon polluted sand and is universally available. Shampoo, washing up liquid and shower gel are universally available and nontoxic. In addition, these consumer goods can be easily applied in remediation. However, in a huge oil spillage, physical and other methods are required in order to remediate the contamination.

5 Chapter 5: Conclusion

Hydrocarbons are naturally occurring and used extensively by society. The refining and use of hydrocarbons can result in increased concentrations in the environment. However, there is also a naturally present background concentration, which depends on location. In the survey chapter 2, the aim was to quantify this background concentration in Saudi Arabian desert sand, which ranged from 234 mg/kg up to 34708 mg/kg for sample Khobar and Karj, respectively.

From an environmental perspective, it is desirable to know threshold levels above which hydrocarbons have a detrimental effect on the environment. While there is some legislation which stated that total petroleum hydrocarbons (TPHs) content range from 10,000 to 50,000 ppm and are considered inhibitory and toxic to most microorganisms in both aqueous and terrestrial environment (US EPA, 2009b; Megharaj *et al.*, 2011), there are few documented laws or guidelines at what concentrations hydrocarbons exceed background levels.

The physio-chemical properties for Saudi sand has been investigated. The results showed that Saudi sands were alkaline in nature as the pH ranged from 8.06 to 9.64. In addition, the sand moisture content was low as they ranged from 0.04% to 0.68% compared to a published study in 2015 (Khan, Hussein *et al.*). The shortage of rain in the Saudi area could be an effective factor of the lower in sand moisture content. Besides, Saudi sand samples had very little organic matter as they ranged from 0.22% to 0.91%. The sand samples were all collected from desert environments and hence the long absence of plant or animal life is reflected in the low organic matter content. It was found that organic matter in sand ranged from 0.21% to 1.75% and 0.01% to 1.03% by Yasir, Azhar *et al.* (2015) and Hashem (1993), respectively.

Mineral contents were found to be extracted in greater quantities using the ashing method rather than aqua regia and nitric acid methods. There was a variation in element concentrations in the Saudi sand samples. . It was found that Ca, Fe, Al, Mg, K, and then Na had the highest average concentrations in all sand samples. Ca in sand samples ranged from 1310 mg/kg to 14823 mg/kg with an average of 7384.7 mg/kg. Fe ranged from 134 mg/kg to 5385 mg/kg with an average of 2044.4 mg/kg. In addition, Al ranged from 1065 mg/kg to 2692 mg/kg with an average of 1651 mg/kg. Mg ranged from 166 mg/kg to 1459 mg/kg with an average of 961.7 mg/kg.

It was possible to fractionate crude oil and diesel into aliphatic and aromatic moieties using activated silica gel chromatographically. The aliphatic fraction obtained using hexane solvent and compounds ranged from C_7 to C_{31} for the crude oil sample and C_9 to C_{27} for the diesel sample. Regarding aromatic fraction, DCM-Hexane (50-50%) was used as a solvent in order to extract aromatic hydrocarbons. Naphthalene, 2-methylnaphthalene, and phenanthrene were obtained for crude oil sample compared to aromatic standard, and there was no peak comparable to the PAHs standard for diesel sample. TPHs was obtained by the combination of aliphatic and aromatic fraction.

The percentage recovery of aliphatic and aromatic fractions in crude oil and diesel was determined. The TPHs percentage recovery was 30% of crude oil. The aliphatic fraction presents 70% while aromatic fraction presents 30% of crude oil. However, the TPHs of diesel percentage recovery ranged from 80% to 90% of diesel. The aliphatic fraction presents 81% while aromatic fraction presents 19% of crude oil. Therefore, approximately 70% of crude oil was not recovered while 10% to 20% of diesel was not recovered. This could be because of 70% of crude oil contains volatile hydrocarbons (under C_{12}), diesel oil contains volatile hydrocarbons (under C_9), polar compounds retained on the column, and possibly asphaltenes (hexane insoluble constituents). The crude oil, which used in this project, its specific gravity was found to be 0.7343 ± 0.0155. At a temperature of 18 ^{o}C. In addition, the API was found to be 61.20, which indicates it is a light crude oil. While, the specific gravity of diesel was found to be 0.8302 at 20 ^{o}C.

Deionised water could be used to extract some hydrocarbon compounds from five Saudi sand samples (Hufuf, Sulayyil, Dammam, Buraidah and Riyadh). There was a variation in hydrocarbons concentration (C_{21} to C_{36}). Samples Hufuf and Sulayyil had the highest hydrocarbon concentration 6651 mg/kg and 1352 mg/kg, respectively. The lowest hydrocarbon concentration was found in sample Riyadh to be 586 mg/kg. Type of sand, type of oil contamination and the amount of rain could have a role in leaching experiment to extract some hydrocarbon compounds.

Organic solvent (DCM 24 h shaking) was used in order to extract TPHs from Saudi sand samples. A range of hydrocarbon compounds was extracted from fourteen Saudi sand samples. There was a variation in percentage of hydrocarbons. It was found that the hydrocarbons from Saudi sand samples extracted aliphatic hydrocarbons from C_{20} to C_{31}. In addition, C_{24} was the highest concentration peak in all samples followed by C_{25}. While the lowest concentration peak found in most samples was C_{21}. Therefore, according to these results, the all samples have the same shape however, different quantities. Sample Karj had the highest TPHs concentration to be 34708 mg/kg. While sample Khobar had the lowest

TPHs concertation to be 234 mg/kg. In addition, it was found that C_{24}, C_{25} and C_{26} were the highest concentration of aliphatic hydrocarbon compounds in all Saudi sand samples. The highest concentration in all samples was found in sample Karj with C_{24} to be 6978.16 mg/kg, while the lowest concentration in C_{24} from all samples was found in sample Khobar to be 53.87 mg/kg.

While hydrocarbons are often complex mixtures, varying in composition as shown in chapter 2, it was desirable to have an assay using an organism's response to the presence of hydrocarbons. Hence in chapter 3, seeds of different plants were used in a germination assay, to investigate their response to varying contamination levels (0.5% up to 10% of crude oil) and 2 climates. In the UK, it was found that lettuce, rye grass and wheat could be an effective plant in remediation up to 2% of crude oil contamination in. These plants could take hydrocarbon from sand by their roots, as they seems to grow better in a contaminated sand than in control sand. The climate conditions affected the roots and shoots growth in contaminated sand, as in cold environment, lettuce, rye grass and wheat grow better than in control sand up to 2% (rye grass) and 10% (lettuce and wheat) of contamination. In a warm environment, lettuce, coriander and wheat did grow better than in control sand up to 2% (rye grass) and 10% (lettuce and coriander).

In the experiment, which was conducted in the UK, it was found that roots of wheat, rye grass and lettuce increased in response to contamination by crude oil. Root length of rye grass decreased at a contamination level of 5 and 10%. However, roots and shoots of radish, cabbage greyhound, cabbage red drumhead and coriander had a decrease at all contamination levels of crude oil. In addition, shoots of wheat showed an increase at all levels of contamination, while shoot length was reduced for all other plants. Rye grass had increase in roots length up to 2% of contamination, and then there was a decrease at 5% and 10% of contamination. Therefore, lettuce, rye grass and wheat could grow in a contaminated sand up normally up to 2% of the contamination. Then after 2% of contamination the roots growth started to be affected in length. Lettuce, cabbage greyhound and cabbage red drumhead all showed a clear decrease in roots length at all contamination levels. These plants seem to be sensitive in a contaminated environment. Therefore, radish, cabbage greyhound, cabbage red drumhead and coriander had a better roots and shoots growth in control sand than in contaminated sand.

However, the same experiment was conducted in KSA indoors with air conditioning 24 hour. The roots of lettuce, ryegrass and coriander increased in response to contamination by crude oil. While radish and wheat had only an increase in roots at 0.5% of contamination. Roots of rye grass had a decrease at 5% and 10% of contamination. Regarding shoots length, only coriander and wheat had an increase at all contamination levels apart from wheat at 10% of contamination. Shoots of radish, cabbage greyhound, cabbage red drumhead, rye grass and lettuce had a better growth in control sand than in contaminated sand. Therefore, in KSA, coriander had an increase in roots and shoots length at all contamination levels while in the UK; had a decrease at all contamination levels. Consequently, coriander seems to grow better in a hot environment rather in a cold environment. In addition, wheat in the UK, had an increase in roots up to 2% of contamination while in KSA had only an increase at 0.5% of contamination. Wheat seemed to prefer growing in cold environment rather than in hot environment.

Plants studied in the UK had a variation in seeds germination percentage among type of plants. It was found that at 0.5% and 1% of contamination, lettuce had the highest percentage of seeds germination among plants, at 100% of germination. While coriander, at 5% of contamination, had the lowest germination of percentage 12% germination.

In KSA, the seeds germination percentage varied for plants at different concentration of contamination. Rye grass and lettuce at 1% of contamination had the highest percentage of germination among plants at all concentrations, with 92% and 90%, respectively. While wheat at 5% of contamination had the lowest seeds germination percentage at 5%. This difference in germination percentage could affect the roots and shoots growth percentage compared to control sand.

When the concentration of hydrocarbons in sands was found too high, a removal procedure involving surfactants and soapy consumer good was investigated. SDS (0.1%) did recover TPHs from contaminated sand with crude oil (2%, 5% and 10%). It was found that at 60 minutes, the SDS recovers more than at 20 and 40 minutes. In addition, the more crude oil contamination the more SDS recovers TPHs. At 10% of contamination level, the recovery was the highest. At 60 minutes, SDS recovered 10.6% of crude oil at 2%. Then at 5% of contamination level, SDS recovered 18% of contamination. At 10% of contamination level, SDS recovered 22% of the contamination. Therefore, by increasing the time and contamination level, the recovery of TPHs increased using 0.1% SDS.

Consumer goods such as washing up liquid, shower gel and shampoo were used in order to remove hydrocarbons from contaminated sand. As the consumer goods were locally available and cheap, this project applied them in the removal of contamination. The parameters of this experiment was 60 minutes stirring, 0.1% consumer goods and 350 rpm. At 2% of contamination, washing up liquid removed 15% of contamination, shower gel removed 12% and shampoo removed 5% of the contamination. While at 5% of contamination, washing up liquid removed 17% of contamination, shower gel removed 17% and shampoo removed 11.5% of the contamination. At 10% of contamination, washing up liquid removed 20.5% of contamination, shower gel removed 18% and shampoo removed 17% of the contamination. Therefore, it was found that washing up liquid removed effectively crude oil contamination from contaminated sand followed by shower gel then shampoo.

In situations where hydrocarbon concentrations are elevated, such as 2% of hydrocarbon contamination it is desirable to have simple means of remediation. This study identified two different approaches with the positive response of wheat and ryegrass to hydrocarbon contamination, and that the use of washing up liquid to reduce it was effective compared to 0.1% SDS solution. These simple means are locally available, cheap and handy. Therefore, washing up liquid could be used in a small level of contamination, as the recovery percentage of hydrocarbon contamination would be approximately 22% of the contamination. Higher contamination levels require multiple washes.

Overall, this research aimed at developing simple tools to assess hydrocarbon contamination and its potential effect on the environment. If it was found detrimental, ubiquitous consumer goods can be used for remediation. An approach has been developed which is low-key and uses locally available materials.

Appendices

Appendix 1: Sand Physio-Chemical properties.

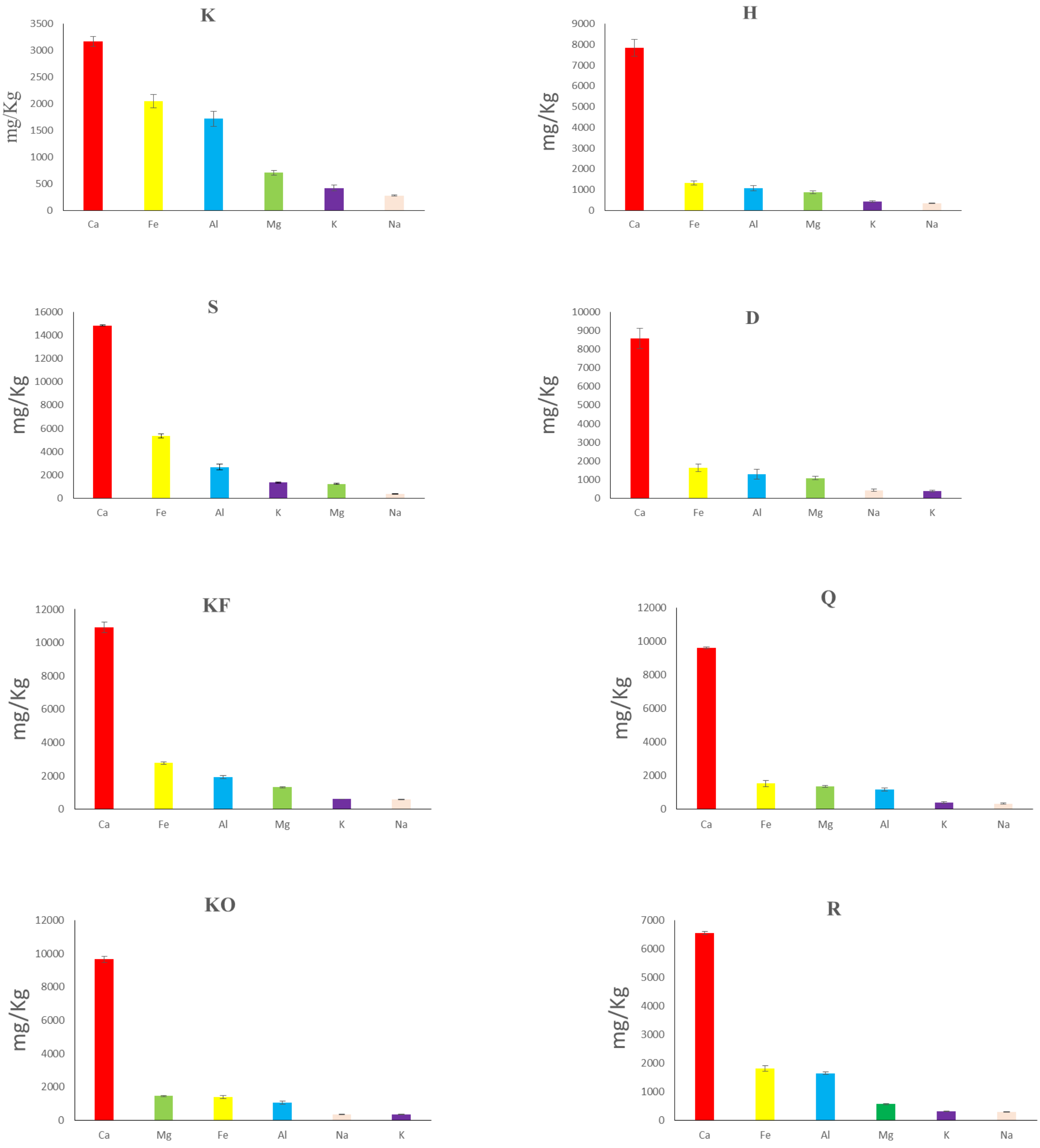
Samples	pH	% Moisture Average	St. Dev.	% Organic Average	St. Dev.	% Ash Average	St. Dev.	% Carbon	% Nitrogen
D	8.7	0.25	± 0.04	0.69	± 0.04	99.30	± 0.06	0.82	0.03
K	9	0.12	± 0.01	0.22	± 0.01	99.77	± 0.01	0.05	0.03
KF	8.1	0.32	± 0.03	0.52	± 0.02	99.47	± 0.02	1.08	0.02
H	8.9	0.13	± 0.01	0.22	± 0.02	99.77	± 0.02	0.49	0.02
S	8.3	0.22	± 0.05	0.87	± 0.05	99.12	± 0.05	3.30	0.03
Q	8.7	0.45	± 0.03	0.45	± 0.03	99.54	± 0.03	0.36	0.02
KO	8.2	0.14	± 0.02	0.45	± 0.09	99.49	± 0.09	0.88	0.02
R	9	0.13	± 0.03	0.39	± 0.04	99.60	± 0.04	0.21	0.02
AN	9.27	0.68	± 0.04	0.47	± 0.07	99.52	± 0.07	0.02	0.003
B	9.64	0.12	± 0.04	0.57	± 0.03	99.42	± 0.03	0.01	0.01
SK	9.37	0.06	± 0.0002	0.32	± 0.03	99.67	± 0.03	0.08	0.01
RF	9.31	0.04	± 0.02	0.35	± 0.05	99.64	± 0.03	0.02	0.01
T	8.39	0.08	± 0.004	0.43	± 0.01	99.56	± 0.01	0.04	0.01
J	8.06	0.15	± 0.009	0.91	± 0.07	99.09	± 0.09	0.05	0.02
N	9.11	0.10	± 0.025	0.65	± 0.03	99.34	± 0.086	0.01	0.01
HS	8.88	0.10	± 0.016	0.86	± 0.04	99.13	± 0.11	2.74	0.01
Average	8.80	0.19	± 0.02	0.52	± 0.03	99.46	± 0.04	0.65	0.02

Appendix 2: Total concentrations of some elements reported as an average (ashing method) value of three replicate measurements ± standard deviation and expressed in mg/kg.

Samples Elements	**D** mg/kg	**K** mg/kg	**KF** mg/kg	**H** mg/kg	**S** mg/kg	**Q** mg/kg	**KO** mg/kg	**R** mg/kg
Al	1334±137	1733±123	1949±123	1065±122	2692±186	1155±42	1075±46	1641±44
Ca	8625±609	3013±162	10943±329	7809±377	14823±48	9563±340	9661±177	6557±52
K	382±35	421±15	608±54	430±47	1386±33	382±30	355±13	317±7
Mg	1064±65	690±24	1313±48	869±24	1236±23	1349±67	1459±46	563±18
Na	434±20	293±38	573±19	340±25	379±20	335±26	357±13	287±12
P	36±11	31±3	49±7	21±2	96±1	45±7	43±1	25±0.4
S	72±12	22±3	250±17	60±7	153±22	88±11	210±29	32±2
Cu	1.2±0.1	1.3±0.1	3±0.2	1.5±0.08	3.7±0.1	1.5±0.2	1.6±0.09	1.5±0.08
Fe	1636±129	1940±100	2773±81	1325±91	5385±89	1533±92	1384±15	1813±89
Mn	58±5	27±5	92±6	32±3	100±5	52±13	44±1.5	24±0.8
Zn	4±0.4	6±0.6	10±0.3	3.5±0.3	13±0.4	10±3	9±2	5±0.5
Sr	43±8	7±1	250±20	20±1	64±8	64±9	64±6	17±0.5
Ba	6±1	11±1	15±0.5	8±0.4	9±1	8±1.2	9±0.5	6±0.1
Si	48±5	30±4	34±8	45±4	74±2	21±1.1	20±2	12±1
Ni	5±1	5±0.8	10±0.6	3±0.3	8±0.9	5.5±1	5±0.1	4±0.1

Samples Elements	**AN** mg/kg	**B** mg/kg	**SK** mg/kg	**RF** mg/kg	**T** mg/kg	**N** mg/kg	**J** mg/kg	**HS** mg/kg
Al	1742 ± 88	2125 ± 76	1238 ± 32	1691 ± 160	2504 ± 96	2887 ± 97	4575 ± 121	2000 ± 45
Ca	1310 ± 18	1543 ± 77	3706 ± 88	1362 ± 59	2302 ± 40	3669 ± 55	2333 ± 72	1471 ± 218
K	55 ± 17	498 ± 23	418 ± 9	302 ± 30	393 ±13	792 ±15	1175 ± 19	814 ±20
Mg	166 ± 7	908 ± 29	689 ±19	364 ± 29	293 ± 50	1069 ± 29	1754 ± 31	2007 ± 35
Na	301 ± 8	270 ± 24	611 ± 12	657 ± 25	662 ± 23	668 ± 44	869 ± 35	637 ± 11
P	42 ± 0.7	43 ± 2	51 ± 3	54 ± 5	192 ± 7	159 ± 4	242 ± 8	106 ± 3
S	29 ± 1	22 ± 1.5	35 ± 2	32 ± 3	105 ± 9	64 ± 5	84 ± 3	126 ± 12
Cu	13 ± 0.2	7 ± 0.3	2 ± 0.02	1.5 ± 0.09	3 ± 0.14	4.5 ± 0.28	7.6 ± 0.4	3 ± 0.07
Fe	134 ± 7	2521 ± 44	1849 ± 98	1357 ± 183	3344 ± 172	4713 ± 175	6839 ± 235	2900 ± 91
Mn	2.38± 0.08	43 ± 2	23 ± 1	15 ± 1.6	80 ± 4	74 ± 2.1	145 ± 5	59 ± 2
Zn	2 ± 0.3	8 ± 3	2.5 ± 0.07	4.5 ± 0.2	8 ± 0.3	10 ± 0.3	17 ± 1	6 ± 0.25
Sr	34 ± 0.4	24 ± 1.7	14 ± 0.2	17 ± 0.7	20 ± 0.3	11 ± 0.3	12 ± 0.4	32 ± 0.5
Ba	13 ± 0.2	7 ± 0.3	2.5 ±0.07	6 ± 0.3	8 ± 0.2	7 ± 0.2	11 ± 0.3	7 ± 0.2
Si	26 ± 5	9 ± 0.9	7 ± 0.5	6 ± 0.1	6 ± 0.1	8 ± 0.8	7 ± 0.4	17 ± 3.8
Ni	0.37± 0.02	5 ± 0.04	1.5 ± 0.06	3 ± 0.2	6 ± 0.38	6 ± 0.2	11 ± 0.6	5 ± 0.1

Appendix 3: Concentration of some major elements in sand samples by ashing method from highest to lowest concentration.



Appendix 4: Aliphatic Hydrocarbons Standard (C_7 – C_{40}).

Carbon Number	Retention time t_R (min)	Compound Identified	MW	Calc RI
7	3.31	Heptane	100	700
8	4.94	Octane	114	800
9	6.77	Nonane	128	900
10	8.54	Decane	142	1000
11	10.19	Undecane	156	1100
12	11.72	Dodecane	170	1200
13	13.16	Tridecane	184	1300
14	14.50	Tetradecane	198	1400
15	15.78	Pentadecane	212	1500
16	16.98	Hexadecane	226	1600
17	18.11	Heptadecane	240	1700
18	19.20	Octadecane	254	1800
19	20.23	Nonadecane	268	1900
20	21.21	Eicosane	282	2000
21	22.15	Heneicosane	296	2100
22	23.05	Docosane	310	2200
23	23.89	Tricosane	324	2300
24	24.61	Tetracosane	338	2400
25	25.23	Pentacosane	352	2500
26	25.77	Hexacosane	366	2600
27	26.26	Heptacosane	380	2700
28	26.71	Octacosane	394	2800
29	27.12	Nonacosane	408	2900
30	27.45	Triacontane	422	3000
31	27.99	Hentriacontane	436	3100
32	28.49	Dotriacontane	450	3200
33	29.04	Tritriacntane	464	3300
34	29.67	Tetratriacontane	478	3400
35	30.40	Pentatriacontane	492	3500
36	31.25	Hexatriacontane	506	3600
37	32.24	Heptatriacontane	520	3700
38	33.42	Octatriacontane	534	3800
39	34.78	Natriacontane	548	3900
40	36.42	T`etratriacontane	562	4000

Appendix 5: Determination of Percentage of Aliphatic and Aromatics in Diesel.

Determination of the Percentage of Aliphatic and Aromatics in Diesel Oil by Gravimetric Methods.

Quantity of diesel oil (mg)	**Volume of diesel (ml)**	**Fraction 1 (mg)**	**Fraction 2 (mg)**	**F1 /(F1+F2) (%)**	**F2 /(F1+F2) (%)**
25.6mg	0.03 ml	**18.93**	**4.25**	**81**	**18**
50mg	0.05ml	**31.47**	**8.32**	**79**	**21**

	F1 Weight (mg)	Average (mg)	RSD (%)	F2 Weight (mg)	Average (mg)	RSD (%)
25.6mg	18.8, 18.9, 19.0, 19.0	**18.93**	**0.5**	4.2, 4.3, 4.2, 4.3,	**4.25**	**1.4**
50mg	31.3, 31.5, 31.6, 31.5,	**31.47**	**0.4**	8.60, 8.30, 8.30, 8.10	**8.32**	**2.4**

Appendix 6: Determination of Recovery Percentage of Aliphatic and Aromatics in Crude Oil.

Determination of the Percentage of Aliphatic and Aromatics in Crude Oil by Gravimetric Methods.

Quantity of crude oil (mg)	Volume of crude oil (ml)	Fraction 1 (mg)	Fraction 2 (mg)	F1 /(F1+F2) (%)	F2 /(F1+F2) (%)
25 mg	0.034 ml	**0.0066**	**0.0030**	**68.64**	**31.35**
50 mg	0.068 ml	**0.0144**	**0.0063**	**69.56**	**30.43**

	F1 Weight (mg)	Average (mg)	RSD (%)	F2 Weight (mg)	Average (mg)	RSD (%)
25 mg	17.38, 13.38, 17.38, 17.38	**17.38**	**0.02**	17.03, 17.03, 17.03, 17.03	**17.03**	**0.003**
50 mg	17.03, 17.03, 17.03, 17.03	**17.03**	**0.003**	17.08, 17.08, 17.08, 17.08	**17.08**	**0.002**

Appendix 7: Determination of specific gravity in crude oil.

Crude oil ml	Average of empty vial	SD	Average of Vial + crude oil	SD	Actual Weight
0.5	7.189425	0.00033	7.5421333	5.774E-05	0.353
1	7.17585	0.000129	7.9050667	0.0003786	0.729
1.5	8.2528	0.0002	9.3295	0.0001	1.0767
2	8.254625	5E-05	9.6746	1E-04	1.420

Water	Average of empty vial	SD	Average of Vial + water	SD	Actual weight
0.5	7.25545	0.0001291	7.747525	0.000125831	0.492
1	7.137225	5E-05	8.104575	0.000125831	0.967
1.5	8.4105	0	9.5046	0.0001	1.0941
2	7.217125	5E-05	9.1644	8.16497E-05	1.947

Weight	crude oi	Water	SG of Crude oil	average	SD	RSD (%)
0.5	0.3527	0.4921	0.7168	**0.7343**	0.0155	2.1129
1	0.7292	0.9674	0.7538			
1.5	1.0767	1.0941	0.7374			
2	1.4200	1.9473	0.7292			

Appendix 8: Determination of specific gravity in Diesel.

Diesel ml	Average of empty vial	SD	Average of Vial + diesel	SD	Actual Weight
0.5	8.6583	0.0003	8.6573	0.0003	0.4113
1	8.4756	0.0001	9.3235	0.0004	0.8479
1.5	8.2731	0.0002	9.5214	0.0001	1.2483
2	8.4245	0.0001	10.1132	0.0002	1.6887

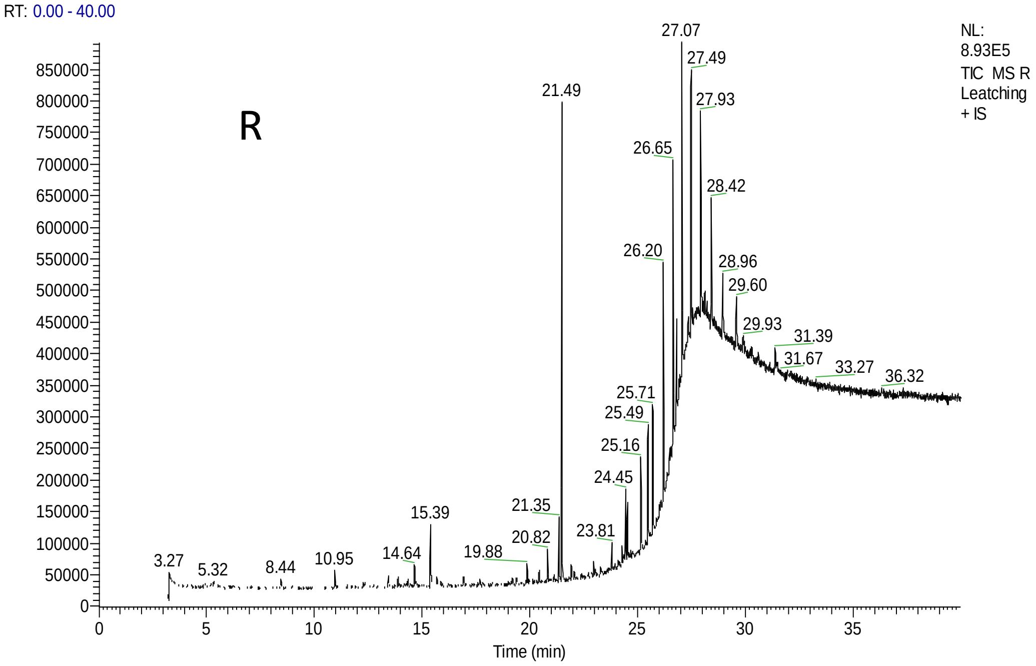
Water	Average of empty vial	SD	Average of Vial + water	SD	Actual weight
0.5	8.4843	0.0002	8.9859	0.0001	0.5017
1	8.3331	0.0001	9.3478	0	1.0147
1.5	8.3103	0.0001	9.8172	0.0001	1.5069
2	8.2951	0	10.3133	0.0002	2.0182

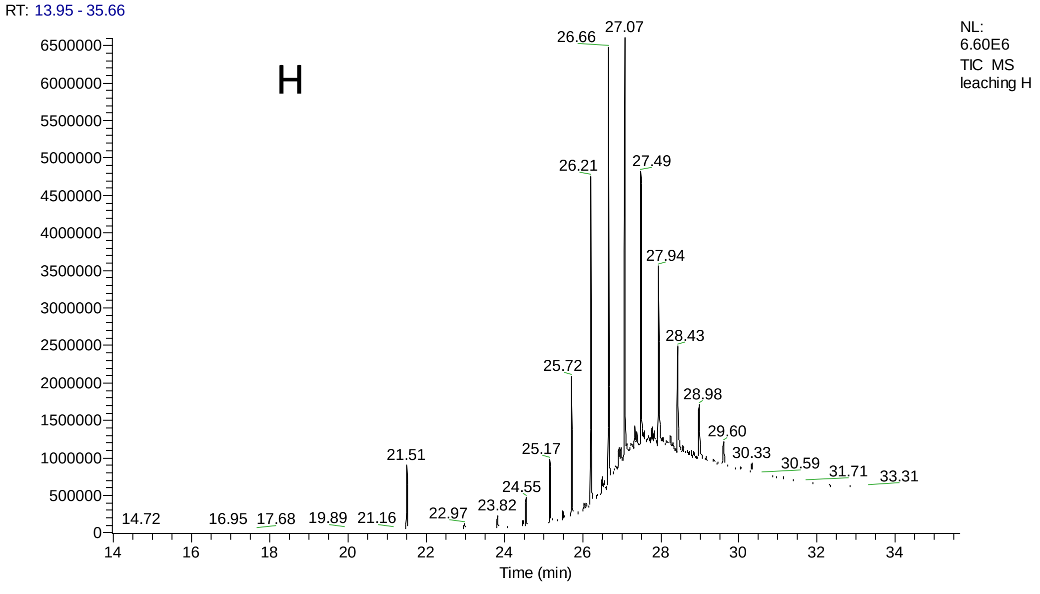
Weight	diesel	Water	SG of diesel	average	SD	RSD (%)
0.5	0.4113	0.5017	0.8179	**0.8302**	0.0078	0.9382
1	0.8479	1.0147	0.8356			
1.5	1.2483	1.5069	0.8284			
2	1.6887	2.0182	0.8368			

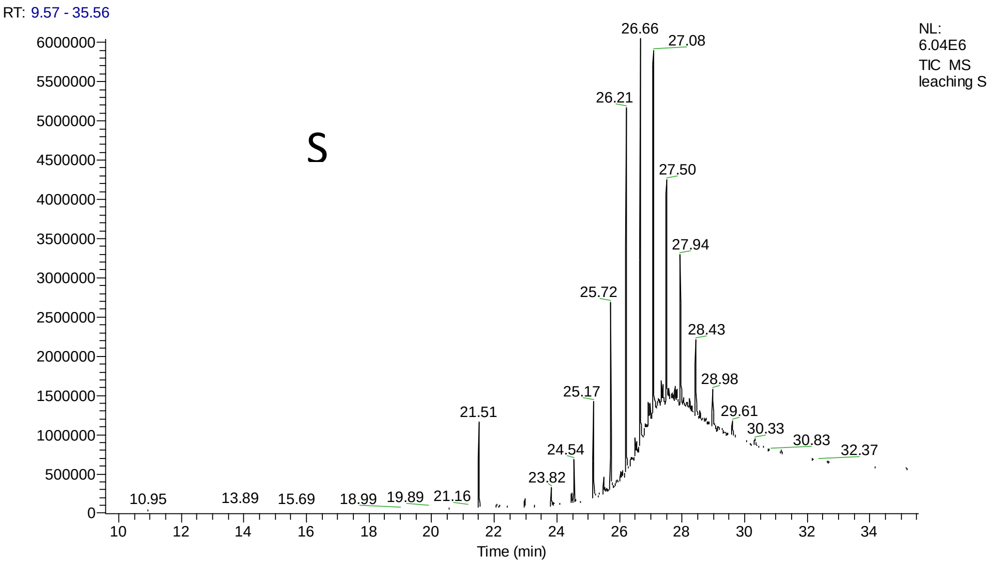
Appendix 9: Quantification of Water Extractable Aliphatic Compounds in the Sand Samples (Leaching Experiment).

	Equation of the line	R^2
C 21	**Y=0.0175X + 0.0104**	**0.9902**
C 22	**Y=0.0175X + 0.0061**	**0.9905**
C23	**Y=0.168X + 0.0062**	**0.9929**
C24	**Y=0.0157X + 0.0062**	**0.994**
C25	**Y=0.015X + 0.00718**	**0.9942**
C26	**Y=0.0141X + 0.1162**	**0.9959**
C27	**Y=0.0134X + 0.1259**	**0.9962**
C28	**Y=0.0127X + 0.1857**	**0.9973**
C29	**Y=0.0121X + 0.1981**	**0.9981**
C30	**Y=0.0116X + 0.1955**	**0.9986**
C31	**Y=0.011X + 0.1914**	**0.9979**
C32	**Y=0.0106X + 0.1756**	**0.997**
C33	**Y=0.0101X + 0.1525**	**0.9949**
C34	**Y=0.0093X + 0.1388**	**0.9937**
C35	**Y=0.0081X + 0.1128**	**0.9923**
C36	**Y=0.0077X + 0.1176**	**0.9912**

Appendix 10: Five sand samples GC-MS chromatogram (Water Extractable Hydrocarbons).



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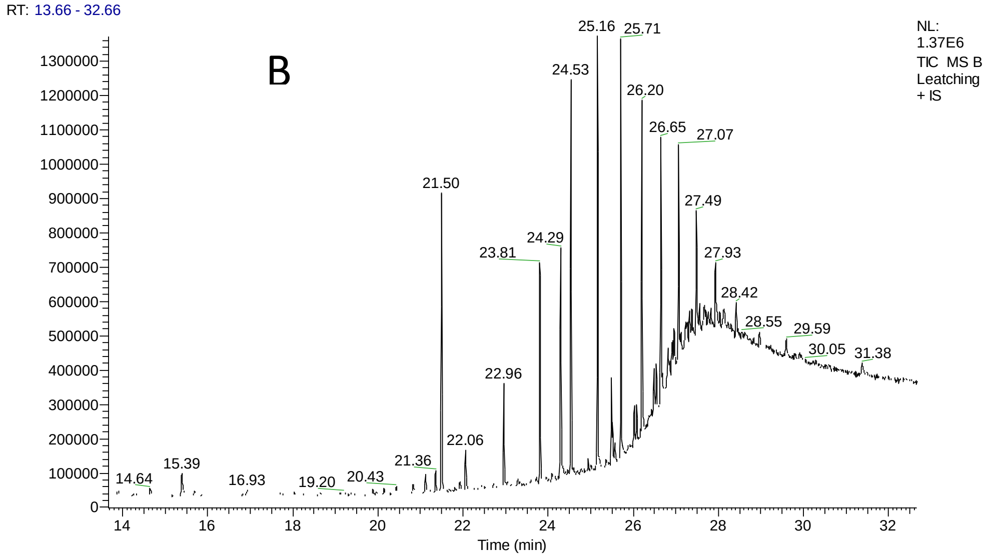
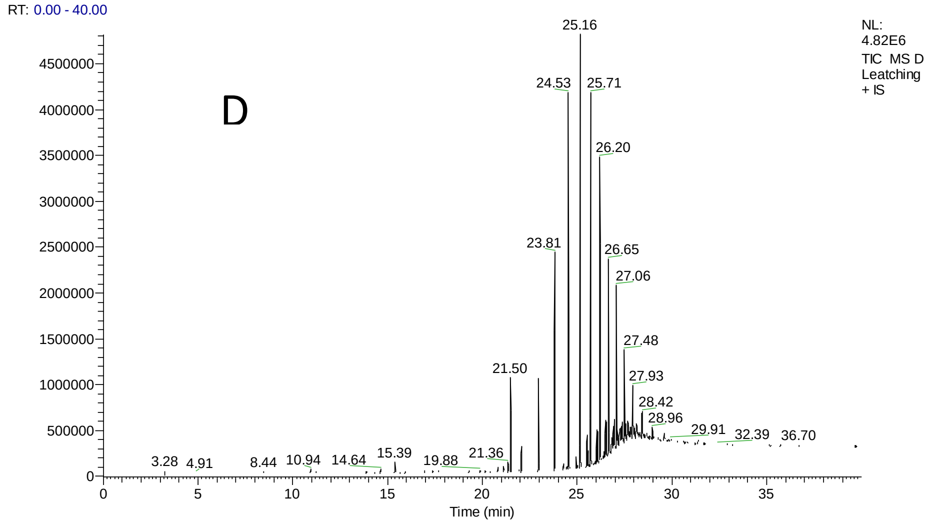
S
NL:
6.04E6
TIC MS
leaching S
26.66
27.08
26.21
27.50
27.94
25.72
28.43
28.98
25.17
29.61
21.51
30.33
24.54
30.83
32.37
23.82
10.95
13.89
15.69
18.99
19.89
21.16
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
10
12
14
16
18
20
22
24
26
28
30
32
34
Time (min)

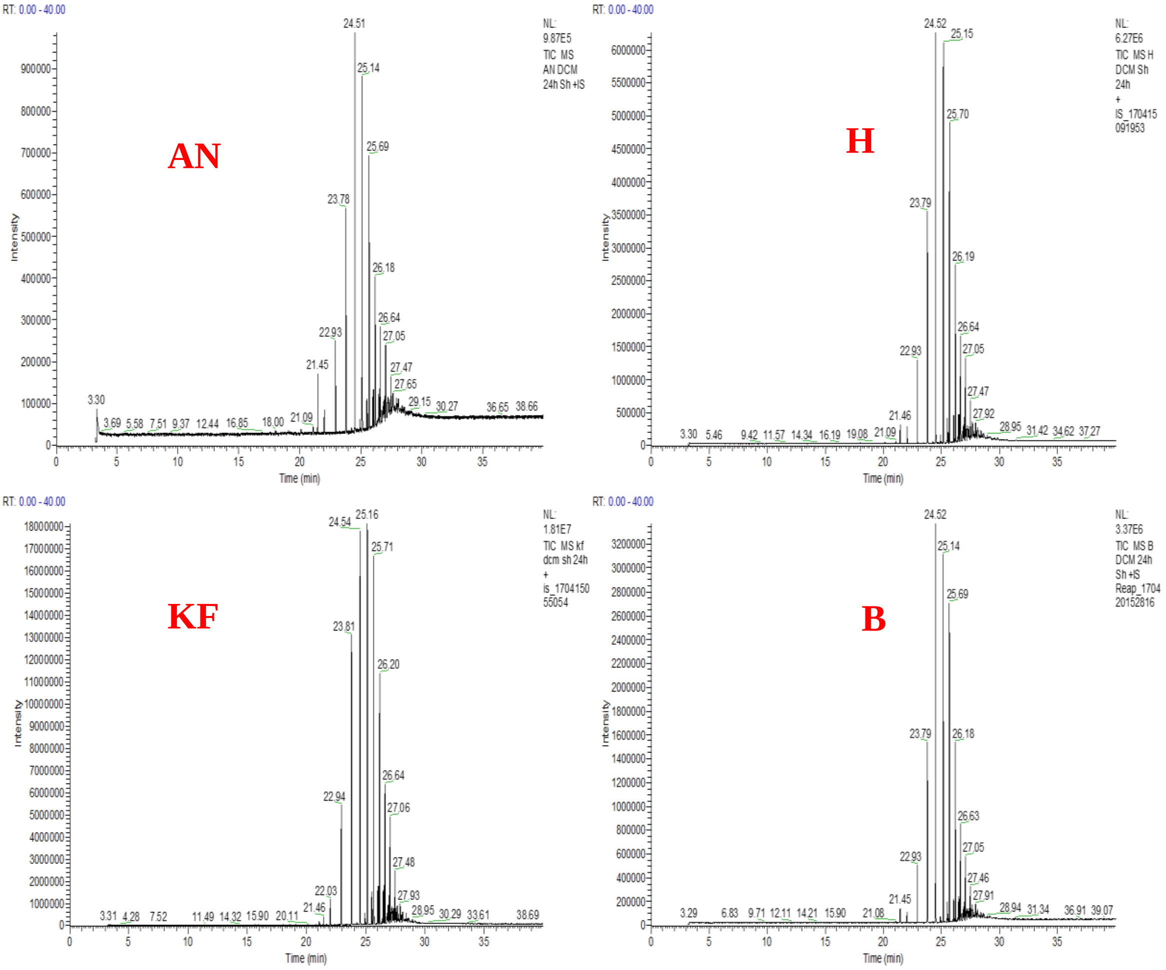

RT: 13.66 - 32.66
B
NL:
1.37E6
TIC MS B
Leatching
+ IS
25.16
25.71
24.53
26.20
26.65
27.07
21.50
27.49
24.29
23.81
27.93
28.42
28.55
29.59
30.05
31.38
22.96
22.06
21.36
15.39
14.64
16.93
19.20
20.43
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
14
16
18
20
22
24
26
28
30
32
Time (min)

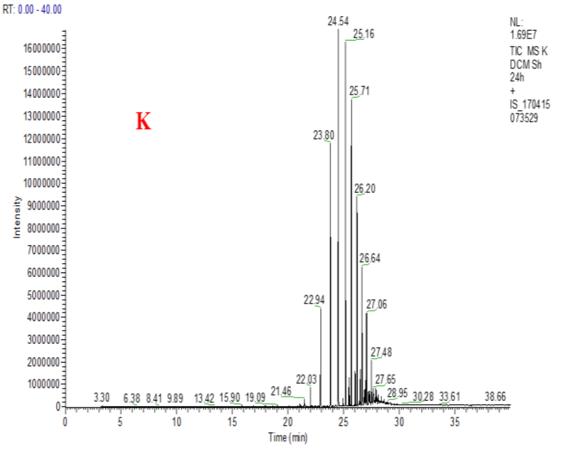

RT: 0.00 - 40.00
D
NL:
4.82E6
TIC MS D
Leatching
+ IS
25.16
24.53
25.71
26.20
23.81
26.65
27.06
27.48
21.50
27.93
28.42
28.96
29.91
32.39
36.70
3.28
4.91
8.44
10.94
14.64
15.39
19.88
21.36
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
5
10
15
20
25
30
35
Time (min)

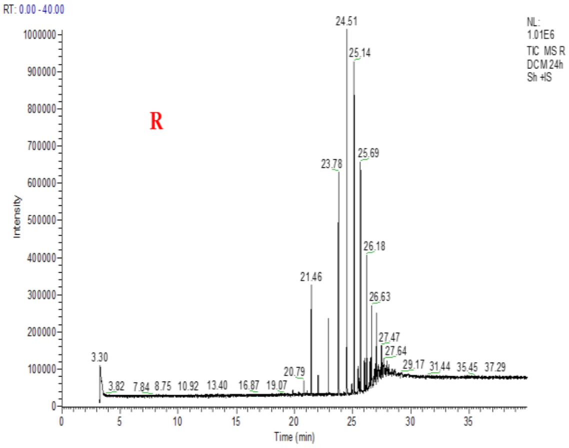
Appendix 11: Concentration of Sand water extractable hydrocarbons.

Sand	**H**	**S**	**B**	**D**	**R**
	mg/kg	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**
C 21	-	-	14.55	29.20	-
C 22	-	-	49.54	79.53	-
C23	27.83	36.53	140.50	200.88	8.64
C24	55.43	67.86	240.04	334.75	10.51
C25	152.43	170.92	249.88	354.04	23.27
C26	329.83	331.78	239.95	321.91	24.09
C27	766.80	660.62	163.98	240.66	63.19
C28	1103.96	857.63	116.02	153.06	96.09
C29	1260.00	987.31	80.77	125.50	203.49
C30	1149.48	619.67	47.49	66.95	101.25
C31	825.48	454.01	9.75	43.20	92.22
C32	481.75	229.31	-		46.73
C33	271.73	162.07	-		6.34
C34	161.86	65.34	-	-	20.76
C35	60.54	22.70	-	-	-
C36	3.48	-	-	-	-

Appendix 12: Sand samples chromatograms obtained from the GC-MS showing range of hydrocarbon peaks.




RT: 0.00 - 40.00
K
NL:
1.69E7
TIC MS K
DCM Sh
24h
+
IS_170415
073529
Intensity
16000000
15000000
14000000
13000000
12000000
11000000
10000000
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
24.54
25.16
25.71
23.80
26.20
26.64
22.94
27.06
27.48
22.03
27.65
21.46
28.95
3.30
6.38
8.41
9.89
13.42
15.90
19.09
30.28
33.61
38.66
0
5
10
15
20
25
30
35
Time (min)


RT: 0.00 - 40.00
R
NL:
1.01E6
TIC MS R
DCM 24h
Sh +IS
Intensity
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
24.51
25.14
23.78
25.69
26.18
21.46
26.63
27.47
27.64
3.30
20.79
29.17
31.44
35.45
37.29
3.82
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13.40
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19.07
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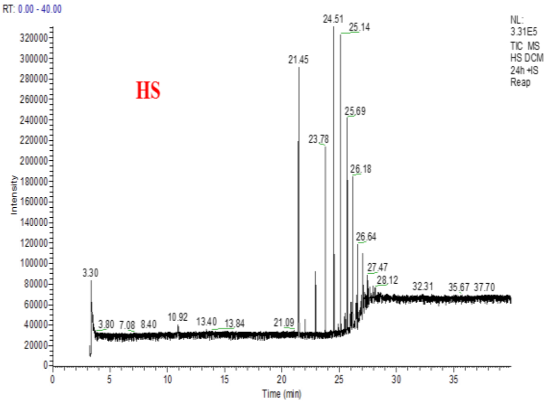
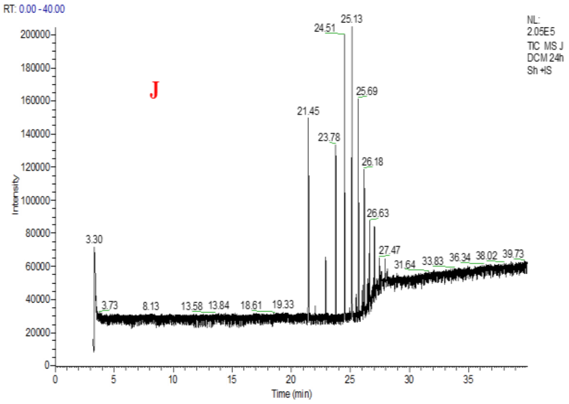
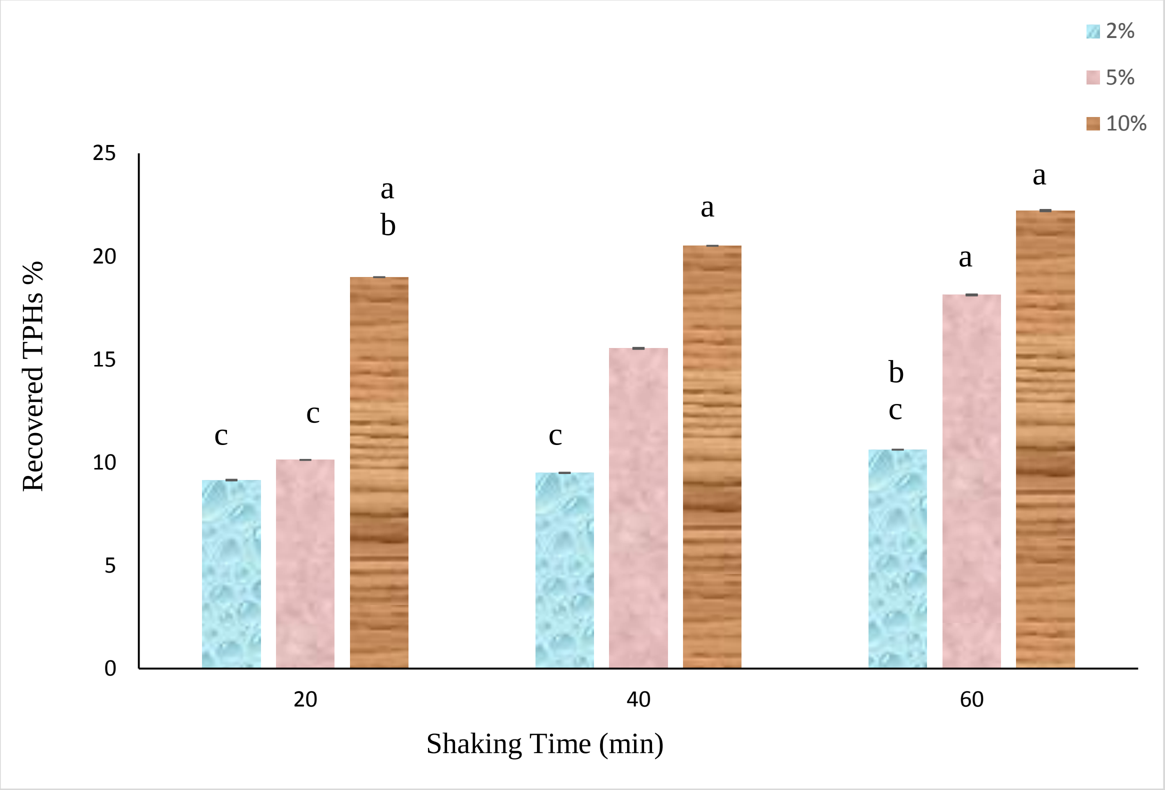
20
25
30
35
Time (min)

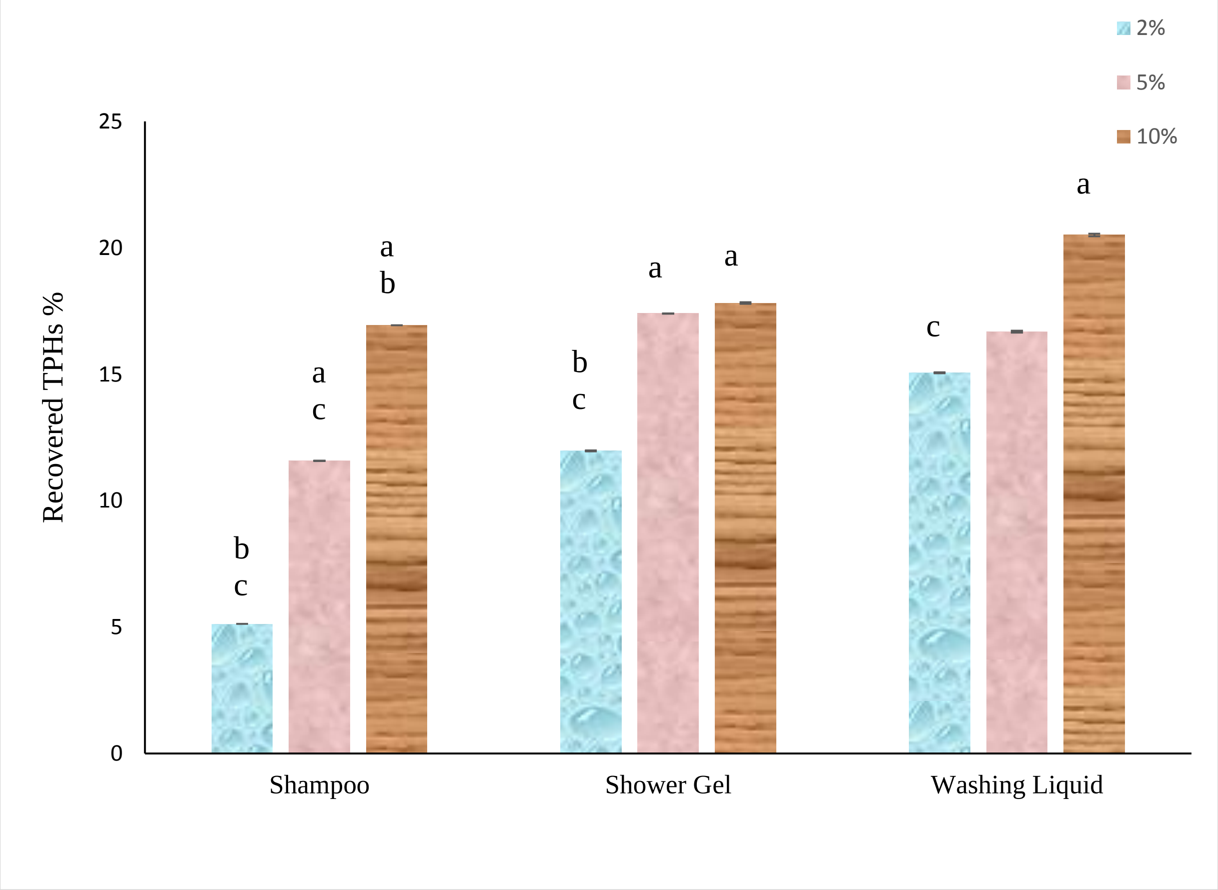

RT: 0.00 - 40.00
HS
NL:
3.31E5
TIC MS
HS DCM
24h +IS
Reap
Intensity
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
24.51
25.14
21.45
25.69
23.78
26.18
26.64
27.47
28.12
32.31
35.67
37.70
3.30
3.80
7.08
8.40
10.92
13.40
13.84
21.09
0
5
10
15
20
25
30
35
Time (min)


RT: 0.00 - 40.00
J
NL:
2.05E5
TIC MS J
DCM 24h
Sh +IS
Intensity
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
25.13
24.51
25.69
21.45
23.78
26.18
26.63
3.30
27.47
31.64
33.83
36.34
38.02
39.73
3.73
8.13
13.58
13.84
18.61
19.33
0
5
10
15
20
25
30
35
Time (min)

Appendix 13: Total petroleum hydrocarbons recovery from three different crude oil contamination levels of control sand at three different shaking time using SDS solution. Bars indicate SEM of three replicates. Lettering a, b and c refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with “a” when compared to the 2 %, “b” when compared to 5 % and “c” when compared to 10 % of crude oil contamination.



Appendix 14: Total petroleum hydrocarbons recovery at three different contamination levels of crude oil using three different consumer goods. Bars Indicate SEM of three replicates. Lettering a, b and c refers to the results of t-tests undertaken where a significant difference ($p < 0.05$) is indicated with “a” when compared to the 2 %, “b” when compared to 5 % and “c” when compared to 10 % of crude oil contamination within each consumer goods.



Appendix 15: Safety Declaration Sheet.

SAFETY DECLARATION

All experimental work undertaken as part of this PhD project have been appropriately risk assessed with the relevant COSSH forms on the CD attached to each copy of this thesis.

Signed ....(candidate)

Date

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