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Characterisation and Bioremediation of Soil Impacted by Libyan Oilfield Produced Water

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Abstract

A large quantity of produced water (PW) is currently produced during crude oil and natural gas exploration and production. The result and effect of discharging PW on the environment has become a significant issue of environmental concern. PW impacted soil is a common environmental problem associated with oil and gas production. This causes the death of plants and contaminates soil. In addition, impacted soil leads to wide spread contamination of surface waters and shallow aquifers.

This work describes an investigation of PW and full characterization of contaminated soils by the disposal of PW at the study site, it includes analysis of both PW and impacted soil using different analytical techniques in order to identify and assay the main constituents that cause the pollution of the soil at the site. The Nasser oilfield, Libya has been chosen as the study site because has a long history of crude oil production since 1956. For this study, six PW samples were collected from the disposal pit bank and through the production stages, eighteen samples of contaminated soil from the disposal pit bank at the study oilfield along with uncontaminated soil samples (taken far from the polluted area) to used as reference. Measurable impacts from PW discharges observed in the soils that have been identified include elevated concentrations of petroleum hydrocarbons and salts in the soil. The total dissolved solids (TDS) concentration in PW and soil can vary between 25407 mg/l to 126065 mg/l, for PW and 20716 mg/kg to 105240 mg/kg for impacted soil. The most common organic contaminants found in PW are total petroleum hydrocarbon (TPH) and BTEX (benzene, toluene, ethylbenzene and xylenes). The average concentrations of TPH for PW and polluted soil samples ranged from 1.2 mg/l to 2.9 mg/l for PW and 10550 mg/kg to 90750 mg/kg for soil samples, BTEX were found in PW at the processes stage and the disposal pit. The average BTEX concentration in PW ranged from 0.11 mg/l to 1.86 mg/l. The polyaromatic hydrocarbons (PAHs) and oilfield chemicals (OFCs) (i.e. corrosion inhibitors, scale inhibitors, biocides and demulsifiers) were also detected in soil and PW at the study site. Understanding the composition of PW and the impacted soil are necessary for assessing the possibility of beneficial reuse and to selecting suitable treatment process for PW and soil. The results showed that the main constituents that impact the soil are hydrocarbons and salts.

In response to a growing need to cleanup environmental contamination, various remediation technologies have been developed to treat soil, wastewater and groundwater contaminated by different pollutants, include *in-situ* and *ex-situ* methods, the choice of the remediation method depends on the type, extent, concentration of the pollutant and the future land use. A combination of method biological, physical, and chemical technologies may be used in combination with one another to decrease the contamination to a harmless and suitable level. Accordingly, to the results an action of soil bioremediation is proposed to remove the hydrocarbons from soil. Bioremediation is one of the most important methods for the

remediation of contaminated soil with petroleum hydrocarbons as it is environmentally friendly and a relatively low cost effective alternative to physical and chemical cleanup options.

Laboratory pilot studies were carried out on bioremediation of soil contaminated with petroleum hydrocarbon from the Nasser oilfield, Libya. Factors that may influence the rate of TPH removed in a crude oil contaminated soil were investigated. Bioaugmentation and biostimulation (a combined treatment) were applied to three soils polluted with approximately, 1 %, 4 % and 8 % of TPH. The bioaugmentation is performed with addition of isolated microbes from soil contaminated with crude oil which were able to grow in a complex mixture of hydrocarbon via inorganic fertilizer (N:P). Biostimulation is performed with addition of different ratio of inorganic fertilizer (N:P) for a period of 240 days. The rates of degradation of petroleum hydrocarbon by the indigenous soil microbe (biostimulation) and in the addition of microbe (bioaugmentation) show that higher degradation rate in all system occurs within the first 60 days. Between 73% to 99% degradation of crude oils in contaminated soils was achieved after 240 days of incubation, depending on the experimental condition. While, in sterile control soil samples biodegradation ranged from 10.90 % to 17.72 %. However, the most rapid biodegradation occurred in the system with the concentrations of 1 % and 4 % and lower biodegradation occurred in the system with a concentration of 8 % TPH, this due to the high concentration of hydrocarbons which could be toxic to the microbes. The information obtained from the results on small scale bioremediation indicates that biological treatment of contaminated soil is appropriate. Identification of bacteria is of great importance for the best understanding of the biodegradation process. In this study, the isolates microbes from the soil were identified as *Bacillus* sp., *Bacillus simplex* strain, *Microbacterium* sp and *Corynebacterium stationis* strain by using molecular biology, based on DNA extraction, genomic analysis of 16S rRNA genes and sequencing.

Abbreviations

API	American Petroleum Institute
ASE	Accelerated solvent extraction
ASTM	American Society of Testing Material
CH	Carbonate hardness
CID	Collision induced dissociation
DAI	Direct aqueous injection
DCM	Dichloromethane
DP	Discharge point
DW	Deionised water
EC	Electrical conductivity
EOR	Enhanced oil recovery
USEPA	Environmental Protection Agency
ESI	Electrospary Ionization
ESI-MS	Electrospary Ionization -Mass Spectrometry
F	Forward
FID	Flame ionization detector
FW	Formation water
GAB	General aerobic bacteria
GAnB	General anaerobic bacteria
GC	Gas Chromatography
GC-FID	Gas Chromatography Flame Ionization Detector
GC-MS	Gas Chromatography Mass Spectrometry
GC-MS-SIM	Gas Chromatography Mass Spectrometry with selective ions mode
GH	General hardness
GOSP	Gas oil separation plant
GPS	Global positioning system
GWA	General water authorities
HAB	Heterotrophic adapted bacteria
HB	Heterotrophic bacteria
HS	Headspace
HOB	Hydrocarbon oxidising bacteria
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IB	Iron bacteria

IR	Infrared Spectroscopy
IRC	Industrial Research Centre
IW	Injection water
LC	Liquid Chromatography
LC-ESI-MS	Liquid Chromatography Electrospary Ionization Mass Spectrometry
LODs	Limit of detections
LLE	Liquid-liquid extraction
LPI	Libyan Petroleum Institute
LPME	Liquid phase micro extraction
LSA	Low specific activity
Max	Maximum
MAE	Microwave assisted extraction
MF	Micro-filtration
Min	Minimum
min	Minute
MPN	Most probable number
MPPE	Macro porous polymer extraction
MS	Mass Spectrometry
MSDS	Material safety data sheets
MSF	Multi stage flash
MW	Molecular weight
NF	Nano-filtration
NOC	National Oil Corporation
NORM	Naturally occurring radioactive materials
NPD	Naphthalene, phenanthrene and dibenzothiophene
OFCs	Oilfield chemicals
OSPER	Osage-skiatook petroleum environmental research
PAHs	Polyaromatic hydrocarbons
PCBs	Polychlorobiphenyls
PDMS	Polydimethylsiloxane
PH	Petroleum hydrocarbon
PID	Photoionization detector
PW	Produced water
QACs	Quaternary ammonium compounds
R	Reverse
RO	Reverse osmosis
SAR	Sodium adsorption ratio

SE	Soxhlet extraction
SFE	Supercritical fluid extraction
SOB	Sulphur oxidising bacteria
SOC	Sirte Oil Company
SPE	Solid phase extraction
SPME	Solid phase micro extraction
SRB	Sulphate reducing bacteria
S/S	Solidification and Stabilization
STDEV	Standard Division
SW	Sea water
T	Temperature
TDS	Total dissolved solid
TEH	Total extractable hydrocarbon
TOG	Total oil and grease
TPH	Total petroleum hydrocarbon
TSA	Total soluble anions
TSC	Total soluble cations
TSPH	Total saturated petroleum hydrocarbon
UF	Ultra-filtration
USA	United State of America
USE	Ultrasonic extraction
VOCs	Volatile organic compounds
ZS	Zoom scans
rpm	revolutions per minute
bbl/d	barrels per day
mbbl/y	million barrels per year
ppm	parts per million
ppb	part per billion
w/v	weight per volume
v/v	volume per volume
meq/l	millequivalents per litre
N:P	nitrogen to phosphorus ratio
ID	internal diameter
Avg	average
m/z	mass to charge ratio
n.d	not detected
Σ	sum
% RED	percentage of reductions

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Petroleum history

1.0 Introduction

Crude oil production is generally considered to have begun on 1859 in Pennsylvania, USA when Edwin L. Drake discovered crude oil and drilled the first deep well to be sunk specifically for oil. Within the year, a further 175 oil wells had been drilled in Pennsylvania and within two years other wells were drilled that produced thousands of barrels per day (Dickey 1959 and Speight 2002). The search for petroleum spread rapidly to the other parts of the world. Small quantities of oil were being produced by 1856 and 1857 in Russia and Romania respectively, and by 1900 this was followed by other countries (Hobson et al., 1975). In Libya oil exploration began in 1955, with the key National Petroleum Law No. 25 enacted in April of that year. Libya's first oilfield was discovered in 1956 and oil exports began in 1961. In 1956 when the first oilfields were discovered they were called the Zelten Oilfield. This is now known as the Nasser Oilfield (Waddams 1980).

Crude oil is a natural product resulting from the decomposition of animal and vegetable remains. The production of a large deposit of fossil fuel requires a large initial accumulation of organic matter, which is rich in carbon and hydrogen. Crude oil is a complex mixture mainly made up from carbon and hydrogen, however sulfur, nitrogen, oxygen and metals (e.g. Fe, Ni, and V) are also found in crude oil. The hydrocarbons in crude oil vary from simple saturated alkanes and aromatics to complex, multi ring structures of high molecular weight (Speight 2002 and Speight 2007). Crude oil is described as containing four hydrocarbons fractions as follows:

- **Saturates:** also called aliphatics, are non-polar hydrocarbons composed of single bonds, including straight-chain and branched alkanes, as well as cycloalkanes (containing one or more rings), which may have several alkyl side chains. Thus, saturates generally are the lightest fraction of the crude oil (Speight 2007).
- **Aromatics:** are hydrocarbons that have at lest one aromatic ring such as benzene and its structural derivates. The aromatics contain alkyl chains and cycloalkane rings, along with additional aromatic rings. Aromatics are often classified as mono-, di-, and tri-aromatics depending on the number of aromatic rings present in the molecule.
- **Resins:** The resin fraction consists of polar molecules often containing nitrogen, oxygen or sulphur. The resin fraction is operationally defined as the fraction soluble in light alkanes such as pentane and heptane, but insoluble in liquid propane (Speight 2007).

- **Asphaltenes:** The asphaltene fraction, like the resins, is defined as a solubility class, namely that fraction of the crude oil that precipitates in light alkanes like pentane, hexane or heptane. This precipitate is soluble in aromatic solvents like toluene and benzene. The asphaltene fraction of crude oil contains the largest percentage of heteroatoms (O, S and N) and organometallic constituents (Ni, V and Fe). The structure of the asphaltenes themselves consists of polycyclic aromatic clusters, substituted with varying alkyl side chains (Speight 2007).

1.1 Crude oil operation and their impact on environments

Oil and gas production operations can be divided into two phases, "upstream oil production" is a term commonly used to refer to the search for and the recovery and production of crude oil and natural gas and is also known as "exploration and production". Downstream production commonly refers to the refining of crude oil and the selling and distribution of natural gas and products derived from crude oil. This includes oil refineries, petrochemical plants and petroleum product distribution. In downstream production areas, hydrocarbons reach the surface and are routed to the central production facilities which gather and separate the produced fluids (oil, gas and water) initially by gravity and capillary forces. The oil production industry typically divides wastes formed in downstream production into three categories: drilling wastes; associated wastes and produced water (E&P Forum 1993&1997and Nagy 2002).

1.1.1 Drilling waste

Crude oil and natural gas explorations encompass activities such as drilling using equipment that can cut through soil and rock in order to identify and access geological formations that contain oil and gas reservoirs. Crude oil or gas wells simply cannot be drilled without a continuous circulation of drilling fluid (or mud) to drilling equipment. The drilling waste is the second largest volume waste generated by the oil and gas industry. Water based drilling fluids may contain viscosity control agents and density control agents. Oil based drilling fluids also contain a base hydrocarbon and chemicals to maintain water in oil emulsions. The cutting rock and soil are lifted to the surface by the fluid circulated through the drilling pipe and usually appear as sludge with an aqueous layer floating on the surface. These are disposed it into open pit nearby the drilling facilities as shown in Figure 1-1. Both the U.S. Environmental Protection Agency (EPA) and the American Petroleum Institute (API) have studied drilling waste streams extensively and identified the constituents that pose the greatest human health and environmental risks (Nagy 2002).

1.1.2 Associated wastes

Associated waste is the small volume of waste generated in combination with oil production. Associated wastes include the oily sludge, emulsions, and workover wastes (including, salt scale, paraffin, acidizing and foam treatment), solids which are collected in surface equipment and tank bottoms, pit waste, acidizing, wastes from dehydration and sweetening of natural gas, transportation wastes, and contaminated soil from accidental spills and release. The sludge waste generally consists of oily sands and untreatable emulsions separated from the production stream, and sediment accumulated on the bottom of crude oil and water storage tanks (Nagy 2002).

Source: Google.

Figure 1-1 A typical drilling waste disposal pit

1.1.3 Produced water waste

Produced water (PW) is any water that is produced to the surface with crude oil and natural gas. It is the largest waste stream source in the entire exploration and production process. Over the economic life of an oil or gas field, the volume of PW produced can be more than the volume of hydrocarbon produced. However, the volumes of PW vary considerably both with the type of crude oil or natural gas production and throughout the lifetime of field. Thus, the cost effective and environmentally acceptable disposal of these waters is critical to the continued economic production of petroleum (Nagy 2002, Joel et al., 2010, Alley et al., 2011 and Ebrahimi et al., 2012).

PW is a complex mixture containing inorganic, organic, production chemicals and natural occurring radioactive materials (NORM). However, most of the water produced can be treated mechanically, chemically and biologically and subsequently re-injected to the subsurface either for disposal or for secondary recovery operations (Nagy 2002,

Joel et al., 2010 and Alley et al., 2011 and Ebrahimi et al., 2012), and would therefore not be an environmental issue.

1.2 The potential environmental impacts

During exploration and the production of crude oil and natural gas a variety of potential impacts on the environment can occur. These impacts depend upon the stage of the process, the size and complexity of the project, the nature and sensitivity of the surrounding environment and the effectiveness of planning, pollution prevention, mitigation and control techniques (E&P forum 1993, 1997 and Dong et al., 2011). However, oil and gas activities occupying large areas involve emissions and discharges of pollutants in all phases from the first seismic surveys until fields are shut down and installations are removed.

In the exploration stage, noise from surveying aircraft and seismic explosions may cause animals to flee from the area. Improper disposal of waste from base camps can lead to contamination of local water and food supplies and environmental degradation. In the drilling and production stage, in cases of improper handling, discharge of waste and toxic substances during drilling can pose a threat to the surrounding environment. Ground water is particularly sensitive to contamination, leading to profound health impacts on wildlife and local people. The most significant source of water pollution during drilling is inappropriate disposal of formation water that is extracted along with oil from the well. This resulting contamination of ground and surface waters can have a serious impact on local people, animals and vegetation. In the decommissioning and rehabilitation stage improper controls can result in soil and water contamination. The potential impacts include human, socio-economic and cultural impact, atmospheric, aquatic, and global impact (E&P forum 1993 and 1997).

1.2.1 Aquatic impact

The principal aqueous waste streams resulting from upstream operations are; first, produced water, second, drilling fluids, cuttings and well treatment chemicals, third, process, wash and drainage water, fourth, sewerage, sanitary and domestic wastes, fifth, spills and leakage. The volume of waste generated depends on the process phase of exploration and production (E&P forum 1993 and 1997).

1.2.2 Ecosystem impact

The areas that are affected by hydrocarbon releases, can recover after the hydrocarbon has been removed, although full recovery can take a number of years. Plant and animal communities may also be directly affected by changes in their environment through variations in water, air and soil/sediment quality and through disturbance by noise,

extraneous light and changes in vegetation cover, these changes may directly affect the ecology (E&P forum 1993 and 1997).

1.2.3 Atmospheric impact

In order to examine the potential impacts arising from exploration and production it is important to understand the sources and nature of the emissions and their relative contribution to atmospheric impacts. The primary sources of atmospheric emissions from oil and gas operations arise from: firstly, flaring, venting and purging gases, secondly: combustion processes such as diesel engines and gas turbines, third, fugitive gases from loading operations and tankage and losses from process equipment. The potential for emissions from exploration activities to cause atmospheric impacts is generally considered to be low. However, during production, with more intensive activity, increased levels of emissions occur in the immediate vicinity of the operations. For instance: flaring of produced gas is the most significant source of air emissions (E&P forum 1993 and 1997).

1.2.4 Oil impacts on global environment

Soil contamination from petroleum hydrocarbons is an important human and environmental health issue around the world. This contamination has probably occurred since the use of petroleum became widespread during the early part of the twentieth century. The potential exists for humans to be exposed to petroleum constituents in soils through various pathways (E&P forum, 1993 and 1997). Potential pathways of exposure to petroleum hydrocarbons in soil depend on the type of soil and petroleum constituents present.

Potential impacts to soil arise from three basic sources: physical disturbance as a result of construction and contamination resulting from spillage and leakage or solid waste disposal. Potential impacts, which may result from poor design and construction, include soil erosion, if native vegetation is removed and soil exposed. Alterations to soil conditions may result in widespread secondary impacts such as changes in surface hydrology and drainage patterns, increased habitat damage, reducing the capacity of the environment to support vegetation and wildlife. Soil contamination may arise from spills and leakage of chemicals and oil, causing possible impact to both flora and fauna (E&P forum 1993 and 1997). However, as the subject of this study is PW and it is effect on the soil a detailed explanation will be given.

1.3 Produced water definition

Exploration and production of crude oil and natural gas usually results in significant volumes of wastes from the facilities of the oil industry such as drilling waste and associated water (i.e. formation water and injection water).

Formation water (FW) is water naturally occurring layers below the hydrocarbon deposits in the porous reservoir media or it is water found in the same reservoir as crude oil and gas. FW (also called brine water or saltwater) is considered to be an essential part of reservoir fluid; this is due to the fact that petroleum deposits are always found in association with FW (Stephenson 1992, Fakhrul-Razi et al., 2009, Joel et al., 2010 and Ebrahimi et al., 2012). FW contains soluble salts to some degree, but the variation in the concentration and nature of these dissolved salts is considerable: waters range in salinity from almost fresh to dense, saline brines. In order to differentiate easily the different types of FW and consistently describe them, it is necessary to define the relevant terms commonly used in the literature. The word "brine" is commonly used to describe any water containing high dissolved salts. However, Carpenter (1978) set out a classification scheme which may be easily applied to FW. It requires that brine must contain over 100,000 mg/l total dissolved salts, water containing between 10,000 and 100,000 mg/l dissolved salts is termed saline, and any water containing less than 10,000 mg/l is either fresh or brackish. By these definitions seawater (35,000 mg/l dissolved salts) is saline, but not brine. Salinity is a measure of the total dissolved salts (TDS) (usually of Na, K, Ca and Mg) in solution, but is often used interchangeably with TDS, which is the sum of all inorganic and organic non-particulate material (Carpenter 1978).

Once the FW is brought to the surface and separated from crude oil and natural gas, this water is called ***produced water*** (PW) and is defined as the water pumped up from an oil well together with oil and gas. In addition, the term PW is also used to describe the water from an oil well after its separation from crude oil using separator equipment. PW is the single largest volume of waste produced by the oil and gas industry (Stephenson 1992, Fakhrul-Razi et al., 2009, Joel et al., 2010 and Horner et al., 2012).

In crude oil and natural gas exploration and production, large volumes of water are produced along with hydrocarbons in crude oil and gas fields all over the world provide largest by-product in the crude oil and natural gas production, this water is mixture of FW and injection water. ***Injection water*** (IW) is the water that is injected into the reservoir to maintain pressure in the reservoir and enhance crude oil extraction efficiency (Thomas et al., 2004, Abdol Hamid et al., 2008 and Horner et al., 2012).

PW is one of the most important sources of pollution associated with crude oil and natural gas production. It contains dissolved solids, high salt content, heavy metals, dispersed oil, dissolved organic compounds (including hydrocarbon), organic acid, phenols, production chemicals and gases (including hydrogen sulphide and carbon dioxide). Besides these constituents, PW may also contain low levels of NORM (Stephenson 1992; Gray et al., 1993, Neff 2002, Thomas et al., 2004 and Ebrahimi et al., 2012). However, the most harmful contaminants present in PW are organic compounds such as benzene, toluene, ethylbenzene, and xylene (BTEX), polyaromatic hydrocarbons (PAHs) and alkyl phenols (Neff 2002, Deriszadeh et. al., 2010, Sundt 2011 and Costa et al., 2012). The discharge of PW containing these pollutants can lead to contamination of soil and aquifers.

1.4 Produced water composition

PW consists of FW (i.e. the water naturally present in the reservoir) and condensed water in the case of natural gas production. In addition, the effluent stream from crude oil production can also contain IW that has been injected to maintain the reservoir pressure. Generally, PW can be characterized as saline water (Fakhrul-Razi et al., 2009). PW usually contains elevated concentrations of organic and inorganic pollutants. PW properties vary considerably depending on the geographic location of the field, the geologic formation with which the PW has been in contact for thousands of years and the type of hydrocarbon product being produced. The injection of additional water into the formation to increase hydrocarbon recovery can also affect the physical and chemical properties of PW (Veil et al., 2004, Abdol Hamid et al., 2008 and Horner et al., 2012). As is mentioned above, PW is a combination of FW and IW containing many pollutants such as organics (dispersed and dissolved oil), dissolved solids, ammonia, boron, heavy metals, radionuclides and OFCs, and hence should be treated before use. As is to be expected most PW are characterized by a high content of petroleum hydrocarbons. PW contains a much higher concentration of dissolved solids than does sea water. Some reservoir is found to contain salinity as high as more than 300,000 ppm, (while the salinity of sea water is 35,000 ppm). The high salinity encountered is mainly due to the presence of dissolved cations (Neff et al., 2002 and Chen et al., 2012). The constituents of produced water (PW) are many and varied, the major components of PW are summarised below:

- Hydrocarbons
  - Dissolved [BTEX]
  - Dispersed

- Precipitated (waxes, asphaltenes)

- Production solid
  - Suspended (including, sands, corrosion and scale products)
  - Dissolved (salts, iron)
    - Major cations (such as Na^+,K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+} and Fe^{2+})
    - Major anions (such as Cl^-, Br^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, NO_3^-, OH^- and PO_4^{3-})
- Production chemicals (or oilfield chemicals (OFCs))
  - Scale inhibitors
  - Corrosion inhibitors
  - Demulsifier inhibitors
  - Biocides
  - etc.
- Dissolved gasses
  - CO_2
  - H_2S
  - O_2
- Trace metals
- NORM (Naturally Occurring Radioactive material)
- Temperature
- pH
- Bacteria

The contaminants in the PW need to be removed before re-use or disposed of (Lu et al., 2006, Joel et al., 2010, Bonfa et al., 2011 and Ebrahimi et al., 2012).

1.4.1 Oil in produced water

Oil is a combination of different hydrocarbons and as well as simple alkanes, alkenes, alkynes and aromatic hydrocarbons include BTEX. NPD, these are 2-3 ring aromatic compounds: naphthalene, phenanthrene and dibenzothiophene, PAHs, polycyclic (3-6 ring) aromatic hydrocarbons [3-6 ring compounds naphthalene and phenanthrene are not defined as PAHs and are included in the NPD group] and phenols. Oil is the common term applied to organic material that is present in PW in suspension or dissolved at the time of disposal. Oil in PW is the sum of that present as dispersed (i.e. small discrete oil droplets suspended in the PW), and that totally dissolved oil in PW (i.e. aliphatic hydrocarbon, low molecular weight aromatic compound, organic acids, phenols and a variety of other substances). Most hydrocarbons are insoluble in water and therefore,

said to be 'dispersed' in the PW. However, some hydrocarbons and dissolved partially in water. BTEX and phenols are the most soluble in water and some of the PAHs are partially soluble and can be present in a dissolved form in water (Tellez et al., 2002 and Ekins et al., 2007).

The oil composition, pH, salinity, temperature, oil/water ratio, type and quantity of oilfield chemicals used and the type and quantity of various stability compounds are the main factors affecting the amount of dissolved and dispersed oil present in PW (i.e. waxes, asphaltenes and fine solids) (Benko et al., 2008 and Fakhrul-Razi et al., 2009). When the dispersed oil is discharged along with PW, dispersed oil may accumulate into the environment, thereby resulting in contamination and accumulation of oil into the ecosystem. Threfore, volatile components may also evaporate into the air. This can potentially affect the environment negatively (Veil et al., 2004 and Wang et al., 2012).

1.4.2 Production solid and dissolved salts in produced water

1.4.2.1 Suspended in produced water

PW usually contain precipitated solids, sand and silt, carbonates, clays and other suspended solids derived from the producing formation and from well bore operations. These solids can influence the fate of PW and its effects, and can reduce the removal efficiency of oil/water separators. In addition, PW contains corrosion and scale products. Scales can form when ions in supersaturated PW react to form precipitates when pressures and temperatures are decreased during production. Calcium carbonate, calcium sulfate, barium sulfate, strontium sulfate, and iron sulfate are the most common scales present in PW. These cause equipment damage, flow restriction and formation damage in addition to lower production rates (Veil et al., 2004 and El-Said et al., 2009).

1.4.2.2 Inorganic in produced water

As is mentioned above, the properties of PW can vary depending on the geological and geographical location and the age of the oilfield. The inorganic pollutants present in PW are primarily derived from the rock formation in which the water has been in contact with it (Benko 2008). Inorganic dissolved compounds in PW include cations and anions, heavy metals and NORM.

1.4.2.3 Cations and anions in produced water

PW contains a wide range of both cations and anions, the major cations are: Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+} and Fe^{2+}. Cl^-, Br^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, NO_3^-, OH^- and PO_4^{3-} are the major anions present in PW. Both cations and anions affect PW chemistry in terms of salinity and scale potential. Salinity refers to the amount of total dissolved salts (TDS) in the water and is frequently measured by electrical conductivity (EC). Salinity is due

to dissolved sodium (Na) and chloride (Cl) with a contribution from calcium (Ca) magnesium (Mg) and potassium (K). Salinity can vary from low to saturation depending on the geology of the area. Sodium adsorption ratio (SAR) is the ratio of the sodium concentration to the combination of calcium and magnesium. It is known to affect on soil dispersibility, another critical parameter for irrigation water (Veil et al., 2004 and Fakhrul-Razi et al., 2009).

1.4.2.4 Produced water salinity

As discussed the salinity of PW can range from very low to high (up to 300,000 mg/l) depending on the surrounding geology and the production process. This is a major contributor to the toxicity of PW (Fakhrul-Razi et al., 2009).

1.4.3 Heavy metals in produced water

Produced water (PW) discharged from crude oil operation usually contains elevated concentrations of trace elements such as cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb), nickel (Ni), silver (Ag) and zinc (Zn) (Moatar et al., 2010). The concentration of such metals in PW depends on the field, particularly with respect to the age and geology of the reservoir formation in which the crude oil and natural gas are produced (Veil et al., 2004 and Fakhrul-Razi et al., 2009).

1.4.4 Naturally occurring radioactive materials (NORM)

Radionuclides in oil and gas production are described as either NORM or low specific activity (LSA) scale. They arise where the geological source rocks have a natural radioactive component. PW generated during the production of oil and gas usually contains enhanced levels of NORM. During the reaction between water and rock in reservoir the NORM such as uranium, radium, and radon are dissolved in low concentration (Fisher 1998 and Moatar et al., 2010). Oilfield FW is usually rich in chloride and this enhances the solubility of other elements such as NORM. These are brought to the surface with the crude oil and must be separated and then disposed. FW that coexists with deposits of oil can have an unusually high concentration of dissolved constituents that build up during prolonged periods of water/rock contact. Furthermore, the scale deposits build up and are accumulated in oilfield equipment. Pipes and tanks that handle large volumes of PW can contain NORM, typically consisting of the radionuclides, radium 226 and 228 (Fisher 1998 and Moatar et al., 2010).

1.4.5 Oilfield chemicals (OFCs) in produced water

The production, recovery and separation of crude oil usually involve addition of various mixtures of OFCs to the oil–water mixtures, to overcome operational problems. These play very important roles in oil production (Grigson et al, 2000 and McCormack et al,

2001). Scale inhibitors, corrosion inhibitors, biocides and demulsifiers are usually added to the top processing equipment of the oilfield to assist separation of produced fluid [oil, gas and water] and mitigate operational problems. Scale inhibitors prevent mineral scale deposition blocking pipe work, corrosion inhibitors prevent pipe work from attack by the salt water and dissolved gases, biocides prevent bacterial degradation of the oil and other products, and emulsion breakers facilitate oil–water separation. Some or all of these OFCs may be discharged to the environment along with the PW (Grigson et al., 2000 and McCormack et al., 2001); the full details of OFCs and their analysis in PW is discussed in Chapter 5 of this thesis.

1.4.6 Dissolved gasses

The major gasses present in PW are carbon dioxide (CO_2) and hydrogen sulphide (H_2S). Natural gas is dissolved in many waters in concentrations approaching saturation values. Dissolved gasses in the PW are generally determined due to their corrosive nature. Oxygen scavengers and other treatment chemicals are available to minimize levels of undesirable dissolved gases in PW (Hansen et al., 1994 and Fakhrul-Razi et al., 2009).

1.4.7 Bacteria

Bacteria are commonly found in both natural and industrial systems. Bacteria are responsible for many problems in the oil industry and may be broadly classified as either attached to surfaces associated with biofilms or planktonic (free floating). This classification may be further refined by considering the main types of organisms likely to be encountered in a PW system. The main types of bacteria are:

- Sulphate reducing bacteria (SRB)
- Iron bacteria (IB)
- Slime formers (include GAB and GAnB
- Sulphur oxidising bacterial (SOB)
- Hydrocarbon oxidising bacteria (HOB)

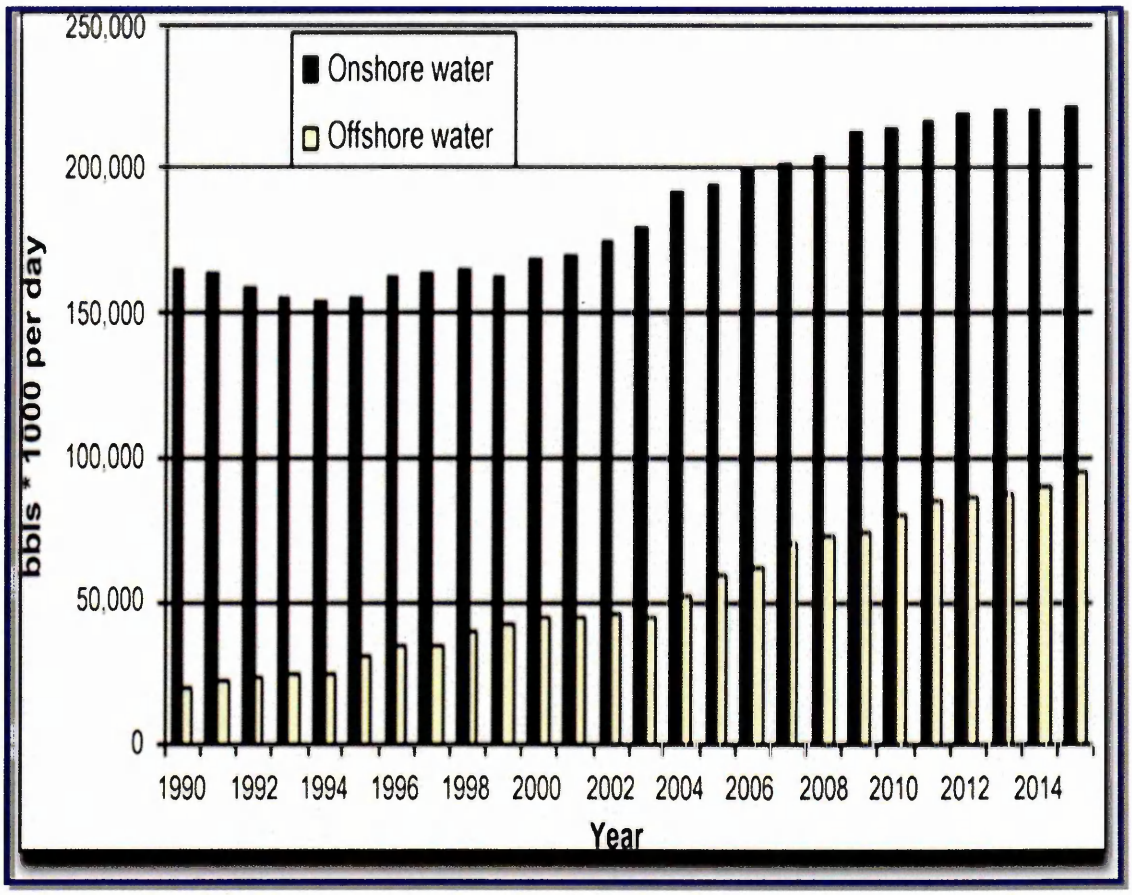
The effects of SRB are the most common biological problems found in oil and gas production facilities. SRB form a physiological and ecological assemblage of diverse types of strictly anaerobic bacteria. They have in common the ability to "activate" sulphate and reduce it to hydrogen sulphide which causes chemical corrosion and fouling of equipment by the formation of iron sulfide. In order to protect the facility from this type of bacterial problem a biocide inhibitor is added to the production fluid (Ayers et al., 2001and Kaur et al., 2009).

1.5 Source and volumes of produced water

The volume of PW generated varies over the life cycle of an oilfield. In the early stages of crude oil production volumes of PW are usually low, relative to the volume of hydrocarbons whereas later PW production volumes continue to increase as the oilfield reaches maturity. The water volume can increase to more than 10 times higher than oil production (as high as 90 %). The increasing volumes of PW, the effects of discharging of PW into the environment has recently become a significant issue of environmental concern all over the world (Lu et al., 2006, Benko et al., 2008, Fakhrul-Razi et al., 2010, Ebrahimi et al., 2012 and Freire 2012). In the United States for onshore production, 7.6 barrels of PW are generated for each barrel of oil produced, while wells elsewhere in the world average 3 barrels of water for each barrel of oil produced (Horner et al., 2011). The PW generated increases as the oilfield becomes older. Figure 1-2 gives an estimate of onshore and offshore PW production since 1990, and forecasts the volume generated up to 2015 (Dal Ferro et al., 2007).

As described above, PW properties and volumes also vary significantly due to the injection of additional water into the formation to increase hydrocarbon production. According to Neff, 2002 the amount of PW discharged from a single platform usually is less than 9,400 barrels/day (bbl/d), whereas discharges from large facilities that process PW from several platforms may exceed a 210,000 barrels/day (bbl/d). Khatib et al., 2003 has reported that in 1999, more than 210 million barrels of PW generated each day worldwide. According to Fakhrul-Razi et al., 2010, the estimated volume of PW generated from oil and gas exploration and production operations worldwide is more than 250 million barrels (bbl) day (with around 80 million barrels (bbl) of oil), this give a ratio of about three barrels of water to one barrel of oil (3:1).

In the United States the PW volume annual estimates for onshore oil and gas wells for the years 1985, 1995, and 2002 were 21, 18, and 14 billion bbl, respectively (Theodori et al., 2011). In addition, Veil et al., 2009, reported that the estimated total volume of PW in 2007 from U.S. onshore and offshore oil and gas production operations was about 21 billion bbl. Furthermore, Benko et al., 2008 has reported that the amount of PW generated from oil and gas productions in United States between 51,157128 to 65,414032 bbl/d this volume of water is greater than the combined daily water consumption for New York City and Los Angeles together. Since, PW is the largest waste-stream source in the whole exploration and production process, a cost effective and environmentally acceptable disposal of these waters is critical to the continued economic production of petroleum (Benko et al., 2008 and Ebrahimi et al., 2012).



Source: Fakhrul-Razi et al, 2009.

Figure 1-2 Global onshore and offshore water production

According to Sirivedhin et al., 2004, about 20–30 billion barrels of oilfield PW are generated annually with the production of oil and gas in the United States. Approximately 65% of this water is beneficially re-injected into petroleum reservoirs for pressure maintenance, 30% is injected into deep wells (saline aquifers) for disposal, and the remaining 5% of PW is discharged to the surface.

Hurst et al., 2005 reported that the volume of PW discharged in the United Kingdom has increased from 1283,121397 bbl/y in 1992 to 2281,104706 bbl/y in 2002 and this has been occurred because production wells became older. The total volume from the Norwegian sector is predicted to reach more than 2096,603590 bbl/y in 2010 (Farmen E, et al., 2010). The total volume of PW discharged into the North Sea in 2002 was over 3270,701601 bbl/y and the volume is expected to increase as the North Sea wells get older (Thomas et al., 2004). Table 1-1 shows the total volumes and discharged volumes of oilfield PW in some country around the world.

Table 1-1 Total volumes and discharged volumes of oilfield produced water in some areas around the world

Oilfield/Country	Total volume [$\times 10^6 m^3$/Yr]	Discharged volume [$\times 10^6 m^3$/Yr]	Reference
North Sea/UK, 2002	390	390	Thomas et al., 2004
North Sea/UK, 2003	400	400	Durell et al., 2006
North Sea /Norwegian, 1992	23	23	Stromgren et al., 1995
North Sea /Norwegian, 2005	147	147	Bioitsov et al., 2007
China, 1998	410	28.3	Lu et al., 2006
USA, 2000	6.1-7.8	6.1-7.8	Viel et al., 2004 and Benko et al, 2008
Marmul Oilfield, Oman 2006	17.5	17.5	Abdel Gawad et al., 2010
Burgan oil field, Kuwait, 1988	10.88	10.88	El-Dash et al., 2006
Gialo oilfield, Libya, 2005	13.07	13.07	Abdol Hamid et al., 2008
Nasser oilfield, Libya,	13.87	13.87	Present Study

1.6 Produced water management

The huge amount of PW generated daily is a challenge for the oil industriy. With volumes of this magnitude, the disposal of PW associated with oil and gas production becomes expensive for the operator and affects the economics of the reservoir and the environment. Produced water (PW) management has been a major concern of the petroleum industry, and continues to affect drilling operations around the world (Mohammed et al., 2009, Li et al., 2010 and Ebrahimi et al., 2012).

The costs associated with PW disposal play a significant role in the economics of the reservoirs. Cost-effective management of produced water improves the productivity of crude oil and natural gas producers by reducing the costs associated with the disposal of produced water. Historically, oilfields PW were managed in ways that were found to be most convenient or least expensive. Over the past decade, oil and gas operators have looked to PW management approaches that minimize the generation of PW and to disposal techniques that offer greater environmental protection and public safety. An example from the oilfield is injection of PW not for disposal but to stimulate secondary production through water flood. Figure 1-3 shows the philosophy of PW management and the options for managing it. One of these options is to minimize the amount of PW that reaches the surface by using a method called downhole separation. The major aim of downhole separation is to avoid handling large quantities of water on the installation by moving the oil/water separation process down into the production well. The separated water is then re injected into a suitable underground reservoir and the oil is pumped to the surface. Using this method the amount of PW can be reduced by more than 50%. Also used are other water minimization approaches and technologies such as mechanical blocking devices or water shutoff chemicals that allow oil to enter the well bore while blocking water flow it, also includes devices that collect and separate PW downhole (Ekins et al., 2005and Veil et al., 2009).

Management of PW is generally expensive, regardless of the price of oil, because of the high quantity of PW that must be lifted to the surface and separated from oil, treated and then injected or disposed off. The cost of managing (handling and disposal) of the PW after it has been already lifted to the surface and separated from the oil or gas at oilfield can range from less than $ 1 to $ 10 dollars per barrel (Boysen et al., 2002 and Altare et al., 2007). While, PW can be reused if certain water quality conditions are met, most PW generated is disposed.

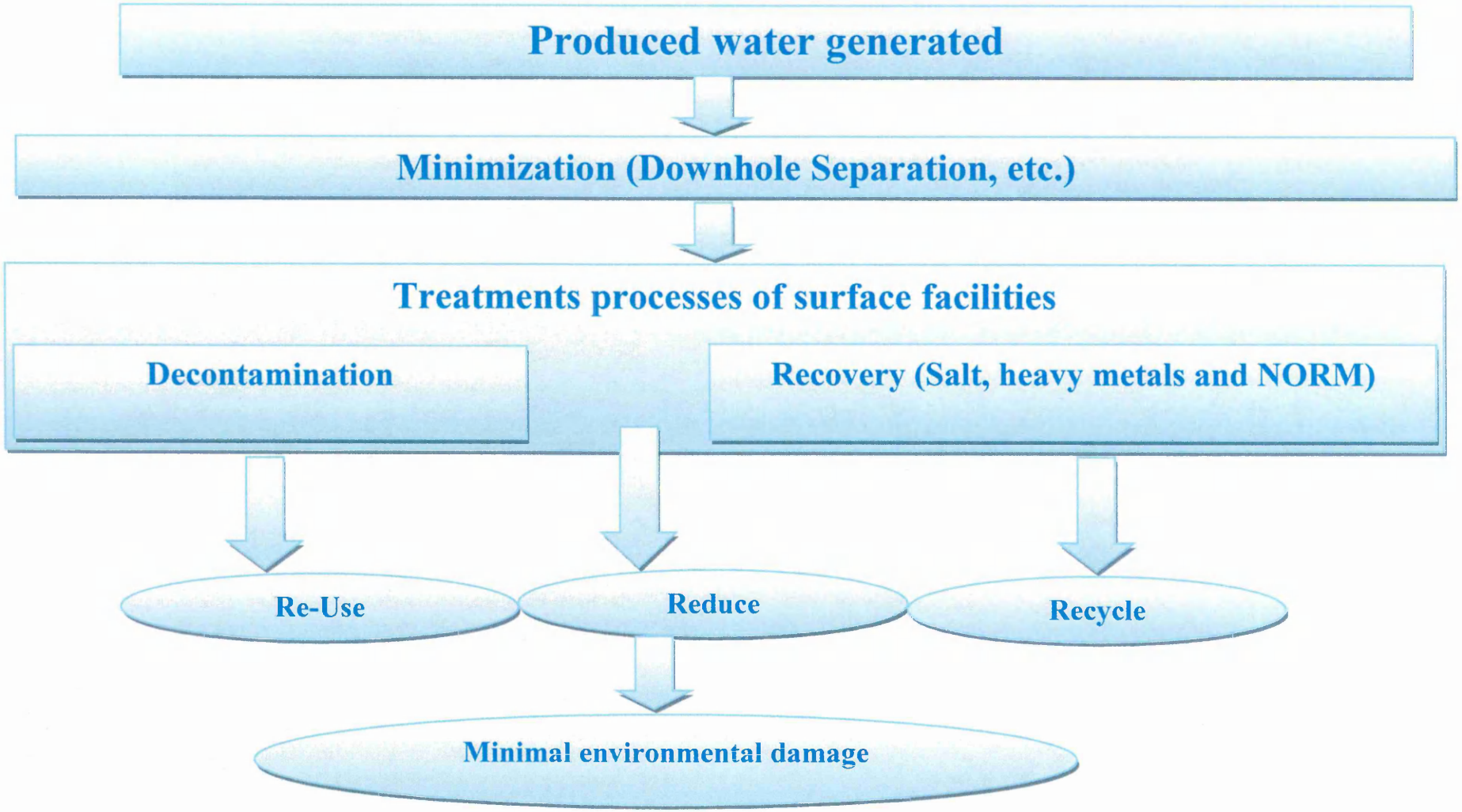


Figure 1-3 Basic produced water management

In oil production activities, PW is managed in a variety of methods. These include: discharge; underground injection for disposal; underground injection for increasing oil recovery; evaporation; offsite commercial disposal; and, beneficial reuse. The remaining PW is disposed through other methods including evaporation and percolation pits (Altare et al., 2007, Kaur et al., 2009, Theodori et al., 2011 and Horner et al., 2012). These management methods are briefly described below.

1.6.1 Discharges

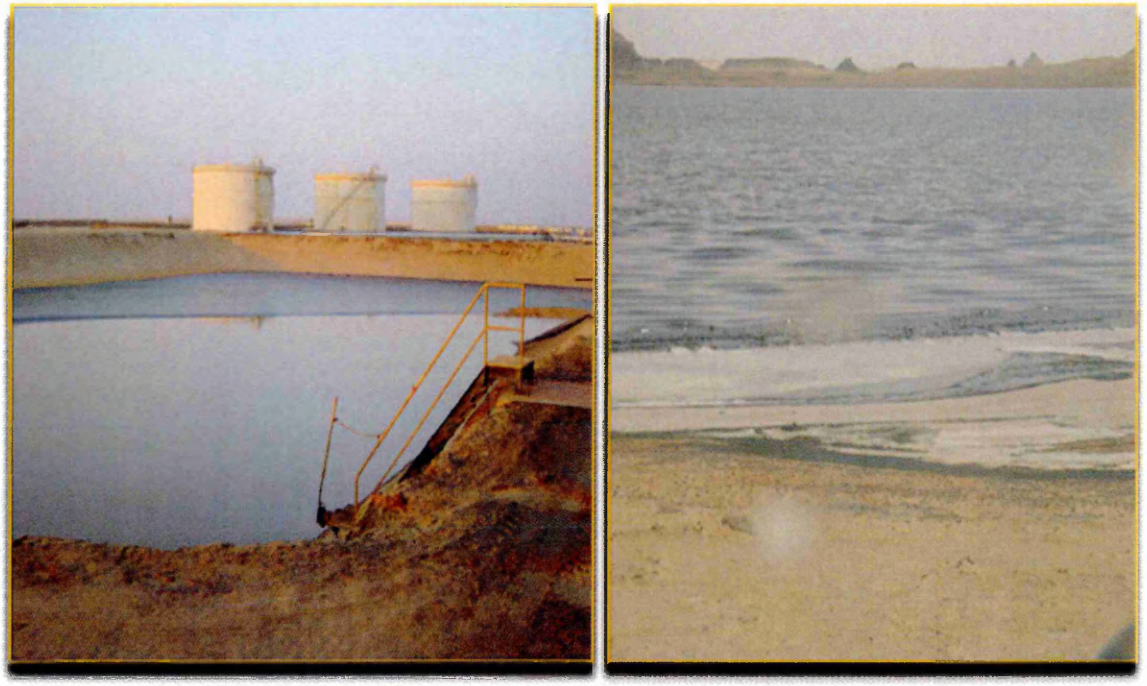
Most onshore oilfield PWs are disposed off at the site of the oilfield. Common practices are the use of a disposal pit or land spreading of drilling wastes and injection of PW. At offshore platforms, most PW and some drilling wastes are discharged to the sea or ocean. Discharge (disposal into the environment, with or without treatment) is usually the preferred option for most crude oil and gas companies due to fact that it involved minimal transportation and treatment. However, there is a need to take more caution than is being done currently because the environmental impacts of discharges are potentially very far-reaching because of the large volumes of discharge involved and the large area impacted. Surface discharge can cause contamination to the soil and ground water (Benko et al., 2008 and Kaur et al., 2009).

There are specific requirements that must be met to comply with environmental regulations before discharge occurs. Strict monitoring should be in place to make sure that the discharge is not harmful to the environment. The disposal of PW into shallow coastal waters and to land has recently become an issue of significant environment concern (Lu et al., 2009 and Bayati et al., 2011)

1.6.2 Evaporation pit (or disposal pit)

After the separation of mixed fluid (oil, gas and produced water) in the surface separation facilities at the oilfield (and due to the extremely high volume of PW present), a first option for the operator company is often surface disposal. In most case, the PW is transported through pipes to be disposed into a large hole, made on the surface and near to the oilfield and left to evaporate and infiltrate this hole is called a surface pit. The size of the pit depends on the amount of PW to be disposed of as shown in Figure 1-4. This method of disposal often results in widespread damage to the surface soil as well as to the ecosystem at the site due to excessive concentrations of different pollutants carried by PW. Surface pits holding PW allow it to either evaporate to the air or percolate into the surrounding soil (Tellez et al., 1995 Kaur et al., 2009 and Wang et al., 2012).

PW evaporated into the atmosphere in vapour form leaves behind solid/ salts in sludge form as shown in Figure 1-5. These pits can only be used when the fluid will not adversely affect surrounding ecosystems including groundwater or surface water. Use of evaporation pits is declining because of potential environmental contamination of groundwater and the potential hazard it posses to flora and fauna by residual oil and salts in these open pits (Veil et al., 2004 and Veil et al., 2009).



Source: Gialo Oilfield, Libya

Figure 1-4 Produced water disposal surface pits

1.6.3 Underground injection or disposal

Subsurface injection is the primary method for the disposal of PW for crude oil and natural gas operations and this method is one of the alternatives to consider where there are waste disposal problems to be solved. PW may be re-injected for disposal to shallower saltwater formations. When it is re-injected for safe disposal, it may be mixed with an underground fresh water source for several reasons. From this point of view, forecasting the pollutant concentrations by knowing the historical data at several locations on a field has great importance when planning the necessary precautions for environmental safety (Okandan et al., 2001). So, the injection wells for disposal must be located in formations that enable water to enter at pressures below the fracture pressure and are isolated from hydrocarbon producing formations (i.e. underground structures that can accommodate the water). Transportation and deep well injection of PW are costly and cause potential contamination to groundwater and thus face strict regulation (Multon et al., 2006).

Source: Images for produced water (Google)

Figure 1-5 Accumulated salts and solid in a produced water disposd pit

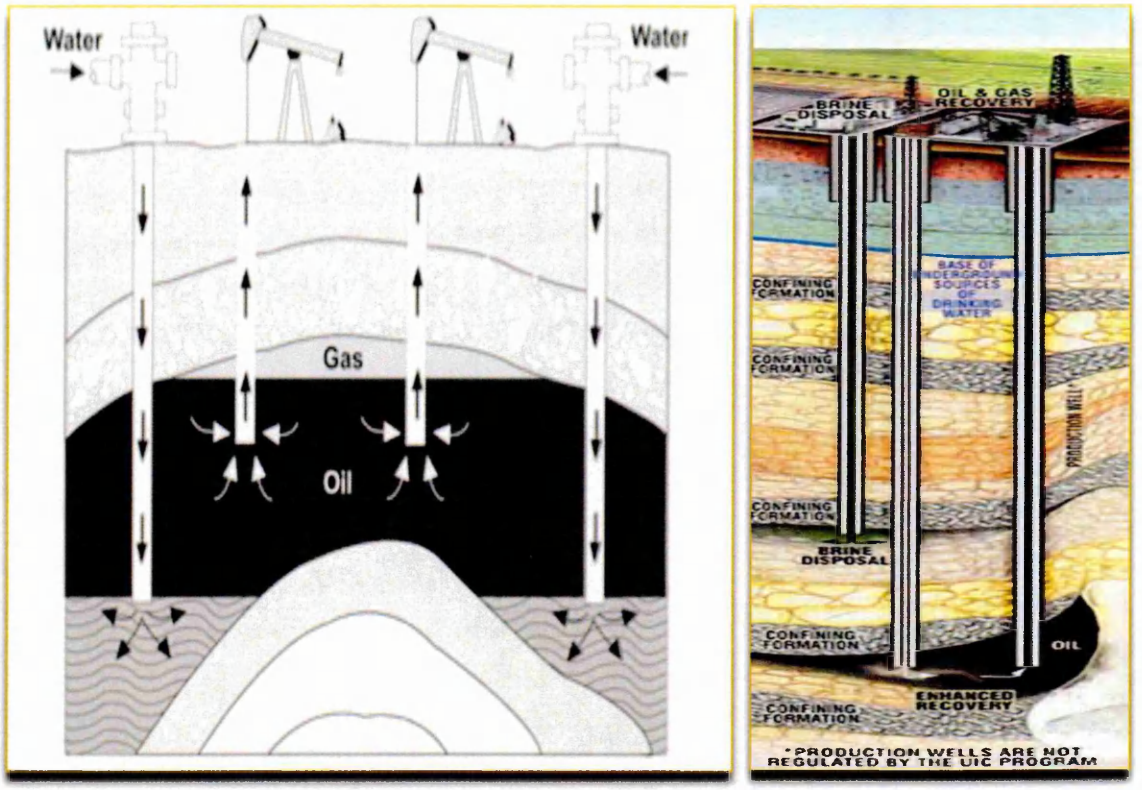
1.6.4 Underground injection for increasing oil recovery

As described produced water (PW) may be re-injected into older, depleted producing formations, to maintain reservoir pressure and hydraulically drive oil toward a producing well as shown in Figure 1-6. This pressure maintenance due to water injection causes high additionally recoverable hydrocarbons. This practice is referred to as enhanced oil recovery (EOR). PW managed by the use of injection for enhanced oil recovery, PW becomes a resource rather than waste products. It is important to ensure that the water being injected is compatible with the formations receiving the water to prevent premature plugging of the formation or other damage to equipment (Lu et al., 2006 and Theodori et al., 2011).

1.6.5 Beneficial reuse

There are numerous management strategies in addition to enhanced oil recovery that use PW for beneficial purposes, such as irrigation, rangeland restoration, livestock, animal consumption, and drinking water for private use or in public water systems, however significant treatment of PW is necessary to met the water quality required. For wildlife or livestock watering or other agricultural uses, excessive levels of sodium can lead to loss of soil structure, reducing the hydraulic conductivity of soils and creating conditions that may limit or prevent plant growth. Additional elements in PW can cause harm to plants when present in sufficient quantities. The use of PW in irrigation with

excessive levels of sodium can lead to loss of soil structure, reducing the hydraulic conductivity of soils and prevent plant growth (Veil et al., 2009 and Veil et al., 2011).



Source: http://en.wikipedia.org/wiki/Enhanced_oil_recovery

Figure 1-6 Schemes of Enhanced Oil Recovery wells (EOR)

PW management today is an important area for the oil and gas industry. Lyngbeak et al., 1991 reported that to handle the ever-increasing amounts of produced water with time; steps need to be taken to improve, upgrade and increase the capacity of PW handling facilities. Evans et al., 1999 discussed several PW handling options and associated dangers. They concluded that disposal, re-injection and treatment of PW are the available options.

Ukpohor et al., 2001 studied the possible negative effect of PW discharged in offshore and onshore areas in the Niger Delta. The authors concluded that the discharge of PW will become a cause of serious environmental hazards to ecosystems and a big problem for the operating companies if effective PW treatment technologies are not employed by the various operating companies to treat the PW. Lawerence et al., 1993 also describe and evaluate a comprehensive study of different scenarios for the cost-effective and environmentally acceptable methods for treatment and disposal of PW. The author concluded that additional surface water discharge opportunities might exist where beneficial use such as irrigation or watering livestock is possible.

Willde et al., 1996 studied the effects of underestimating the production rates and quality of produced water in oilfields in Oman. This led to complications as higher

water injection rates than expected were required for subsurface disposal of PW as more PW had to be disposed of than capacity and these affected the economics of the PW injection wells and oil production from the field. To reduce the amount of PW to be re-injected into the ground hence saving on PW disposal costs. Over the past years the oil industry has looked into ways of using this PW for beneficial uses. New technologies, especially in the area of membranes, are available for waste water and sea-water desalination and these may be applied to PW treatment and desalination for beneficial use (Gurden et al., 2000).

Miller et al., 1997and Doran et al., 1998 carried out pilot plant studies on converting PW to beneficial use quality. The system of treatment consisted of deoiling, dissolved organic removal, and partial demineralization. Settle et al., 1998 studied the use of PW in irrigation or other beneficial purposes by looked into ways of using plants to remove salt and other elements from the PW. Gurden, et al., 2000 looked into ways of removing contaminants from PW using reed bed technology, in Oman, and using this water for agricultural purposes.

1.7 Produced water treatment

The handling and disposal of the PW is one of the greatest environmental challenges to crude oil and natural gas exploration and productions since it is associated with organic and inorganic materials. Crude oil and natural gas companies need to treat or dispose of PW according to recent strict regulations. In general the main stream coming up from the well head (or reservoirs) consist of crude oil, natural gas and PW. The first step is the mixed fluid is separated through separation process, and then the separated crude oil is pumped into the storage tank. Thereafter the oily PW is pumped to the treatments plant for further treatment. The basic PW treatment steps are shown in Figure 1-7.

The PW handling costs have increased extremely over the past two decades and will continue to increase due to PW quantities increasing (Pendashteh et al., 2010, Fakhrul-Razi et al., 2009 and Ebrahimi et al., 2012). Figure 1-8 shows the PW treatments technologies targeting removal of different elements. PW treatment is an effective option for PW handling. To determine and suggest any technology for PW treatment, initially, is necessary to understand the physical and chemical composition of PW to have an idea which treatment methods can be applied for different type of PW. However, treatment will be required to met beneficial use effluent standards; in the majority of applications.

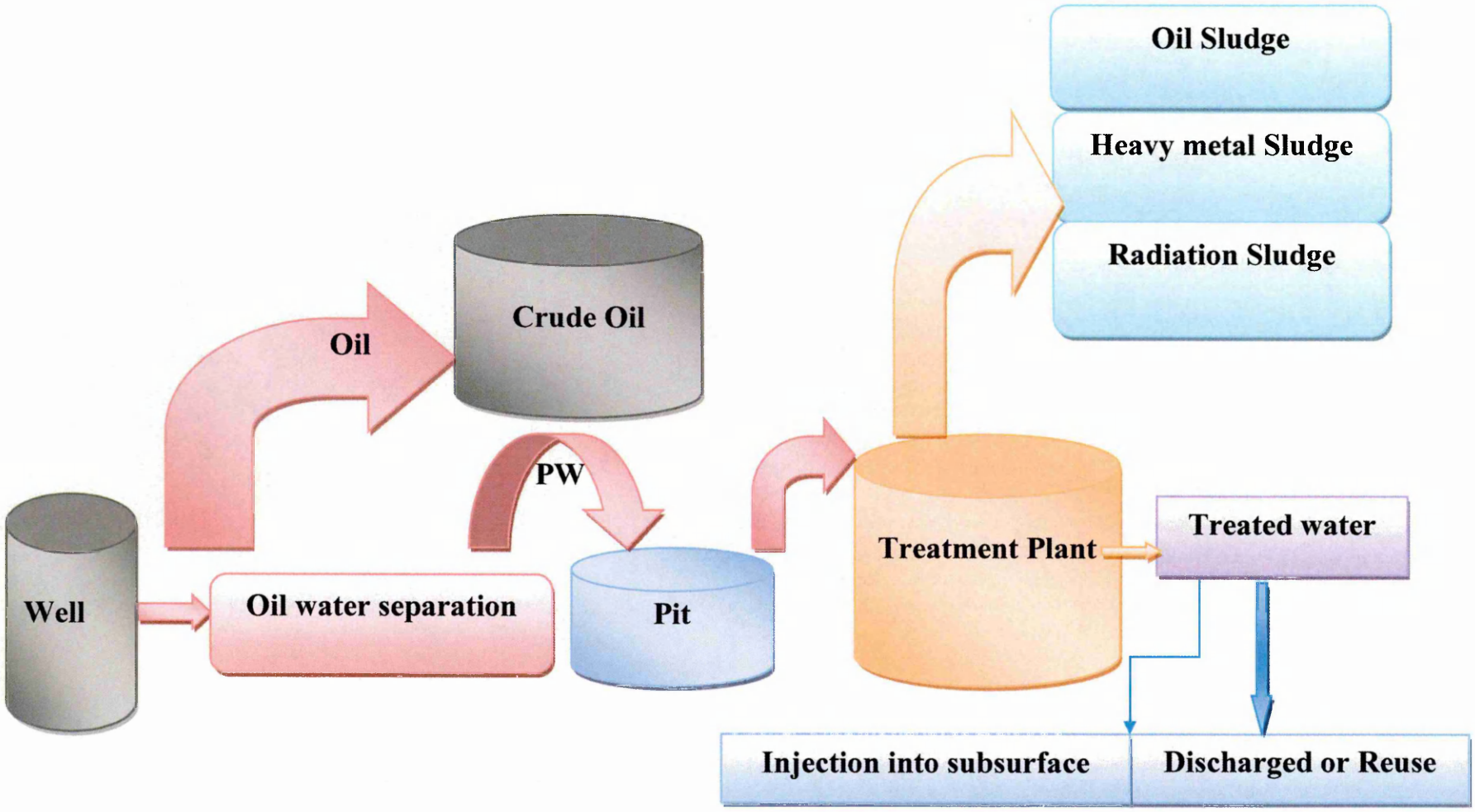


Figure 1-7 Produced water treatment steps

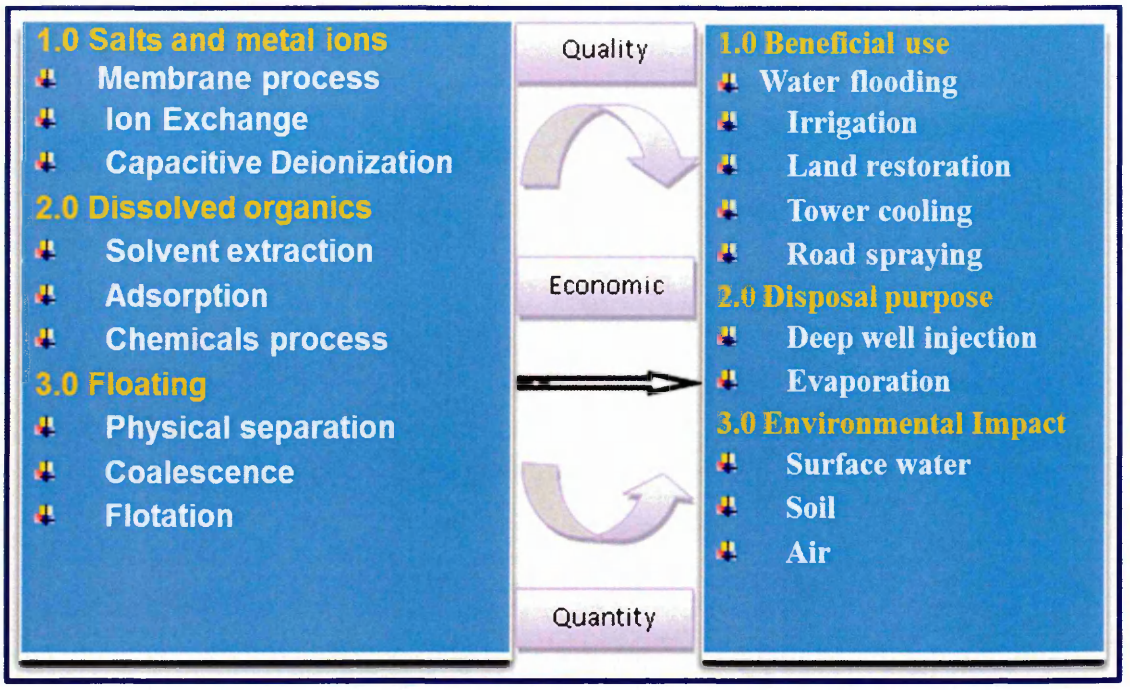


Figure 1-8 Strategies of produced water purification methods and beneficial uses

Produced water (PW) treatment processes have been commercially used for the past couple of decades in the oil and gas industry. New technologies have developed in the field of oil-water separation and desalination that can be used to remove the contaminants from PW. Treatment is focused on the removal of dissolved and dispersed oils and greases, scale control, and suspended solids and reducing the volume of water produced. However, with more stringent regulations and the current focus on reuse of PW, for example in irrigation, livestock watering, groundwater recharge, and habitat restoration, greater attention is being paid towards new and innovative treatment processes with even greater capability to remove the contaminants present in PW. In addition to oil and grease and suspended solids removal, treatment objectives now highlight the removal of organic compounds such as BTEX from PW.

The general objectives for the oil sector in the treatment of PW are as follows (Fakhrul-Razi et al 2009):-

- Removing dispersed oil and grease from PW (called De-oiling).
- Soluble organic removal.
- Removing suspended solid (particles and sand).
- Dissolved gases removal (such as CO_2, H_2S).
- Removing microorganisms (called disinfection).
- Desalination to remove dissolved salts.
- Softening: removing excess water hardness.
- Removing NORM

Physical, chemical, mechanical and biological treatments are the conventional methods used for the treatment of PW. Gravitational separation, centrifugation, coagulation, flotation, filtration and adsorption combined with biological treatment are the conventional methods used for removal of free and emulsified oil and suspended solids and also BOD removal from PW (Bayati et al., 2011). PW treatment can be divided into two groups:-

- Mechanical: includes separation by using different equipment such as filters, separators, hydrocyclones, coalesces, centrifuges, membranes, skim tanks and gas flotation units
- Chemical treatment (which may be required for a variety of reasons):
  - ✓ In the primary separation the reverse emulsion breaker or demulsifier is added to assist the separation of oil emulsions from the PW.
  - ✓ To increase the efficiency of the media filtration process by addition of flocculants upstream
  - ✓ In the primary separation process the scale inhibitor is added upstream to minimize scale formation.
  - ✓ Biocides are added to minimize bacterial growth (OSPAR 2002).

To improve the water quality for disposal or beneficial use many technologies are used. These include reverse osmosis (RO), distillation, electrodialysis, desalination and ion exchange methods. Air stripping, activated carbon adsorption, synthetic zeolite adsorption, membrane filtration, wet air oxidation and biological treatments methods can be used to remove dissolved organics from PW. Due to the complex chemical composition of PW each technology has its limitations. Different technologies are favoured around the world for wastewater treatment. Such technologies include micro-filtration (MF), ultra-filtration (UF), reverse osmosis methods and ion exchange methods for de-mineralization purpose. Hydrocyclones, membrane separators, centrifuge, air-floaters, emulsifiers, and adsorbers have all been used to remove oil and grease from the PW.

Different combinations of methods may be used to remove pollutants from PW (Fakhrul-Razi et al., 2009, Bayati et al., 2011, Dong et al., 2011, and Ebrahimi et al., 2012). The most widely used first stage of PW treatment is based on gravity separation of oil from PW.

Different equipment is used to perform this process. Filtration or barrier separation can be employed but this method is not suitable for dissolved oil. Dissolved oil is removed using filtration or membrane methods. Along with physical and chemical methods, biological treatment methods can be used when the flow rates are high. Biological treatment is attractive for remediation of dissolved oil in PW due to its low cost (Bayati et al., 2011 and Wang et al., 2012). In general, oily PW treating equipments are categorized as:

- Primary treatment equipment to protect the downstream facilities is directed at the removal of water from crude oil. It is based on a gravity separation process using a skimmer tank based on the difference between the specific gravity of oil and water. When the retention time is sufficient, oil floats to the surface and can be separated by an overflow, this technique is suitable only for non-dissolved components.
- Secondary treatment equipment: Parallel plate separators consisting of parallel plates, gas flotation and hydrocyclones are used to remove oil droplets from produced water. A hydrocyclone is based on centrifugal forces and the difference between specific gravity of oil and water.
- Tertiary treatment equipment: gas flotation technology using gas bubbles to remove very fine oil particles in which fine oil droplets are attached to the gas bubbles and as result a gas and oil are driven to the surface. The treatment of the dissolved hydrocarbons can be removed by using membranes and biological treatment (Ekins et al., 2005 and Li et al., 2010).

Many separate and combined physical, chemical and biological methods are available for PW treatment such as:

1.7.1 Macro-porous polymer extraction (MPPE)

Macro-porous polymer extraction (MPPE) technology was introduced in 1990 and is used to remove dissolved and dispersed hydrocarbons from water. The MPPE technology is based on the liquid- liquid extraction process. In this technique the polluted produced water is passed through MPPE. In the process of MPPE the hydrocarbons are condensed and then separated from PW under the process of gravity. By using MPPE technology 100% of hydrocarbons are removed and the treated water can be re-used (Ekins et al., 2005 and Fakhrul-Razi et al., 2009).

1.7.2 Activated carbon

Adsorption can be accomplished using a range of materials, including zeolites, organoclays, activated alumina, and activated carbon. The adsorption techniques include

activated carbon filters with regeneration by wet air oxidation and oil-adsorbing media canisters based on resins, polymers and clay technologies. Organic compounds and some heavy metals in PW adhere to porous media or carbon surfaces. After a few runs, the wet air oxidation process can regenerate activated carbon. Activated carbon can remove soluble BTEX (Ekins et al., 2005 and Fakhrul-Razi et al., 2009).

1.7.3 Reverse osmosis (RO)

Reverse osmosis (RO) technology is a separation process used to remove organic contaminates, dissolved solids and bacteria from water, RO is very effective and can be used to treat brine (PW) with high salinity. The pressure force the water molecules through the membrane while leaving the larges molecules behind, i.e. it uses pressure in excess of osmotic pressure to force a solvent through a membrane that retains the solute on one side and allows the purified solvent to pass to the other side. The RO process is based on the reversal of water flow from high salinity to the permeate stream on the opposite side of the membrane (Ekins et al., 2005 and Fakhrul-Razi et al., 2009).

1.7.4 Multi stage flash (MSF) process

The Multi stage flash (MSF) process is a water desalination process that distils salty water by converting the water into steam in multiple stages. In MSF process the water is passed through different stages and because of the reduction in atmospheric pressure the water is continuously boiled and flashed into steam. Each stage is equipped with tubes of heat exchangers, where the flash water is condensed and collected as clean water (Ekins et al., 2005 and Fakhrul-Razi et al., 2009).

1.7.5 Ion exchange

Ion Exchange describes the separation, purification and decontamination of liquids by the use of solid ion exchangers. The technology is one of the more flexible treatment options because it is able to treat water of varying quality. Ion Exchange methods are based on a reversible chemical reaction in which positively or negatively charged ions in PW are exchanged for another ion, with similar charge attached to the solid particles. Ion exchange technology can be used to removal heavy metals and hardness and other elements from PW. The quality of water treated with this method is high enough to be used for irrigation, livestock etc. (Ekins et al., 2005 and Fakhrul-Razi et al., 2009). Tellez et al., 2002 has conducted a pilot field study to evaluate the performance of using activated sludge treatment system for removing TPH from PW at South Western US oilfield. They conclude that the treatment system is capable of removing hydrocarbons from PW. Fakhrul-Razi et al., 2009 have reviewed the technologies used for oily PW treatment. They conclude that no single technology can meet suitable effluent

characteristics; hence two or more treatment system might be used. In addition, choice of the best technology is depend on PW composition.

Fakhrul-Razi et al., 2010 studied the performance of a membrane sequencing batch reactor and a reverse osmosis process for treating PW. The author found that the membrane sequencing batch reactor inoculated with isolated microorganisms played an important role in the biodegradation of the pollutants and also found that it was feasible to treat the PW using RO and that the quality of PW met the requirements for discharge or re-use. Melo et al., 2010 conducted a pilot scale RO and nano-fitration (NF) membrane system treatment of PW from an oilfield in the northeast of Brazil. They aimed to assess the quality of treated water for reuse for irrigation or other beneficial uses. The authors concluded that by using RO/NF effectively resulted in decreasing conductivity and TDS and the quality of PW met the requirements for beneficial use. The authors also concluded that it is important by study the water toxicity in order to meet the guidelines for irrigation use.

1.8 Impacts of produced water discharges

PW can present a threat to aquatic life if discharged into surface soil; it penetrates to water tables or to crops when the water is used for irrigation. The fate and effect of PW in the environment varies greatly, depending on the volumes of PW, the nature, characteristics and constituents present in the effluent and the type of production. Gas and oil operations give rise to significantly different produced water characteristics. PW has various potential environmental effects depending on where it is discharged (Ekins et al., 2007). Discharge to the open ocean has a lesser effect because of the dilution that take place compared to discharge made to a small stream or surface soils. The disposal of PW and other aqueous effluents that arise from oil production has always been a problem in remote locations, whether in the desert or in the jungle. In areas with high biodiversity, environmental considerations have been for many years a significant factor in the design of effluent treatment systems. However, desert environments have been seen as being less worthy of preservation (Veil et al., 2004 and AbdolHamid et al., 2008). This has led to the proliferation of the evaporation pond, effluent pit, soak-away or settlement pit as the primary means of effluent disposal. All pollutants in PW either individually or collectively, when present in high concentrations, can affect the ecosystem. Numerous studies have been conducted on the fate and effects of PW discharges in the coastal environments, and these studies have shown that PW can contaminate sediments and such contamination correlates directly with the PW

discharge quantities, type and the concentrations of pollutants present in PW (Rabalais et al., 1992, Henderson et al., 1999 and Benko, et al., 2008).

1.9 Produced water disposal at Libyan oilfields

The disposal of PW can have serious negative effects on water sources and livestock, potentially harming soil and farming communities. Acute events such as surface disposal and spills, can cause loss of life and do extensive damage to local environments. Since the start of oil production in Libya and up until the 1980s, most of the oilfields were operated within the frame of joint venture agreements with foreign companies. The philosophy adopted by these companies was to produce crude oil at the maximum rate at the lowest cost and to maximize their revenues. The disposal of PW became a problem at the late stage of depletion of these oilfields when the volume of PW reached a high level at some Libyan oilfields (e.g. Nasser oilfield, Abuattifel oilfield, Intisar oilfield). In Libya, most oil and gas operating companies dispose of PW by disposing it into an open pit near to the oilfield and leaving it to evaporate, penetrate through the soil column. Only a small amount of PW was re-injected for enhanced oil recovery. Figure 1-9 shows the produced water disposal methods at the Libyan oilfileds. The Sirte Oil Company (SOC), Libya, generates more than 315,000 barrels of PW daily from the Nasser oilfield. This PW is directly discharged to an evaporation pit. It is felt that much of this oilfield PW could be treated and reused by oil and gas industry.

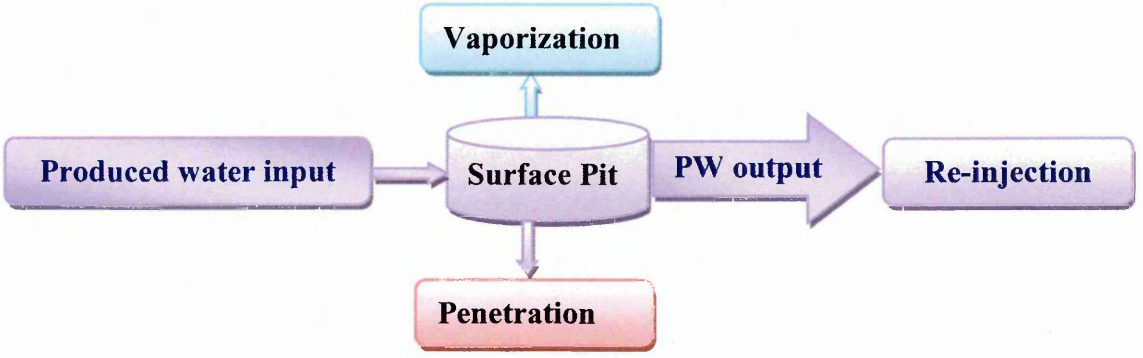


Figure 1-9 Produced water disposal methods at Libyan oilfields

1.10 Produced water impacted soil

Depending on the chemical composition of the PW and the nature of local environment, salt and hydrocarbon associated with such releases have the potential to impact soil, vegetation and water resources. PW impacted soil is the most common environmental problem associated with oil and gas production. Exposure to PW causes the death of plants and contaminates soil and surface waters and aquifers (Li et al., 2006 and Abdol Hamid et al., 2008). During soil pollution by PW, the salt load primarily affects the organic horizons and can sometimes reach the groundwater level. When discharged to

the surface environment into an open pit it will adversely affect or destroy surface soil and can penetrate through the geological column to contaminate water tables. High salt and hydrocarbons contents impact soil and change in soil properties such as

- Soil structure.
- Soil physical structure by coating soil aggregates
- Soil water holding capacity
- Air and water movement in the soil matrix
- Reducing and diverting water infiltration into the soil
- Obstructing air and water movement
- Reduced bioactivity
- Reducing cation/anion ion exchange on soil aggregates
- Increased water runoff and erosion of soil (Suleimanov 2005).

The disposal of PW into the surface soil will result in a range of direct and indirect environmental impacts. Ecosystem sensitivity to brine contamination and the ability to recover depends on the flora, fauna, soil properties, geology, slope, hydrology and climate of the spill site. Moreover, the spill quantity and composition influence ecosystem responses. According to Harris et al., 2005 Americium Petroleum Institute (API) published a field manual in 1997, regarding the remediation of soil impacted by saline PW. This handbook describes three separate remedial activities. The first, excavation of the contaminated soil is utilized when the brine components represent a significant threat to nearby surface water. The second activity involves the addition of gypsum to improve the structure of the topsoil and encourage the downward movement of brine components through the soil profile. The third action employs plants capable of surviving in highly saline soil, to stabilize the topsoil. It is important to understand the long-term and short-term effects of PW and hydrocarbon releases from these sites in order to develop risk-based remediation plans.

Remediation is particularly needed in aging and depleted fields where land use is changing from petroleum production to residential, agricultural or recreational uses. In response to a growing need to address environmental contamination, numerous remediation technologies have been developed to cleanup contaminated sites by petroleum hydrocarbon, including *in-situ* and *ex-situ* methods. Among one of these treatment methods is soil washing which is a promising modern remediation technology due to its potential for treating soils contaminated with petroleum hydrocarbon and also those contaminated by heavy metals (Urum et al., 2004).

1.11 Overview of petroleum contaminated soil treatment technologies

The exploration and production of crude oil generate differents sources of pollution into the soil, water and air. Petroleum hydrocarbon contaminants are typically present at the site of a release in more than one phase (vapour, liquid and dissolved and/or adsorbed). Soil heterogeneity and other difference in the subsurface make every site different. Remedial technologies that are effective for removing petroleum hydrocarbons that exist in one phase may not work for another phase. In addition, remediation systems applied at one site may not work at a different site. A particular contaminated site may require a combination of procedures to allow the optimum remediation for the prevailing conditions. Biological, physical, and chemical technologies may be used in combination with one another to remove or reduced the contamination to a safe and acceptable level (David 1996 and Khan et al., 2004). Remediation (clean-up) of a contaminated soil by crude oil and petroleum fractions is an important issue in today's news. Huge amounts of money are being spent to clean up contamination once it has occurred (Millioli et al., 2009). Over the past decade, a number of different technologies or combinations of technologies have been used to remediate petroleum-contaminated soil. The remediation approach can vary significantly depending on site-specific factors such as type, volume and extent of contaminates. Surface soil contamination, typically involves more conventional approaches to the remediation of petroleum hydrocarbon contaminated soil including vapour extraction, bioremediation, soil washing extraction, and natural attenuation methods. When hydrocarbon affected soil extends to deeper depths, the cost of physical removal for subsequent treatment is costly (David 1996 and Khan et al., 2004). Selected alternative treatment technologies used for petroleum contaminated soil that may apply *in-site* and *ex-site* are discussed below:

1.11.1 Bioventing or soil vapor extraction

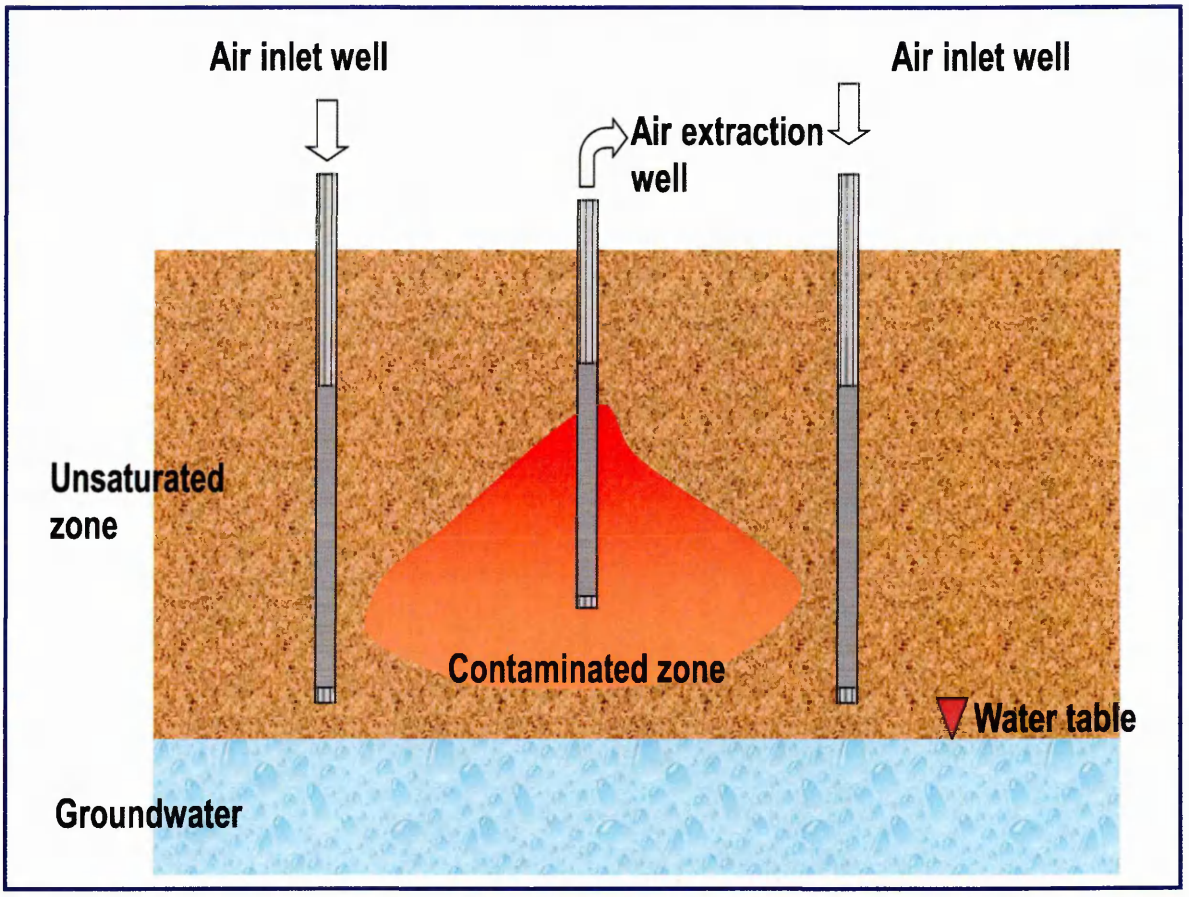
Bioventing also known as soil vapor extraction or vacuum extraction is an *in-situ* remediation method and an accepted recognized and cost effective technology for treating the unsaturated zone (i.e. soil above the water tables) which is contaminated with hydrocarbon such as volatile organic compounds (VOCs). Bioventing is a method that uses naturally occurring soil microorganisms to degrade hydrocarbon adsorbed in soil in the unsaturated zone by providing oxygen to them. In bioventing the air at different rates is pumped through the well installed at the contaminated area (Khan et al., 2004 and Magalhaes et al., 2009). The VOCs are withdrawn through the extraction well by a vacuum pump and then the vapors are treated by carbon adsorption before being released to the air. The increased air flow rate to subsurface soil (i.e. the polluted area)

gives sufficient oxygen for the microbes to break down hydrocarbons, especially those that are less volatile.

Magalhaes et al., 2009 have studied and applied a combination of an air-injection bioventing system with other methods for the treatment of contaminated soil. The authors concluded that a combination of the methods used proved to be an efficient solution for the treatment of soil contaminated with toluene as it promoted high removal of up to 99 % of the contaminant reducing both the soil contamination and the outlet gas emissions, when a high air flow rate was applied. A schematic diagram of the bioventing method is shown in Figure 1-10.

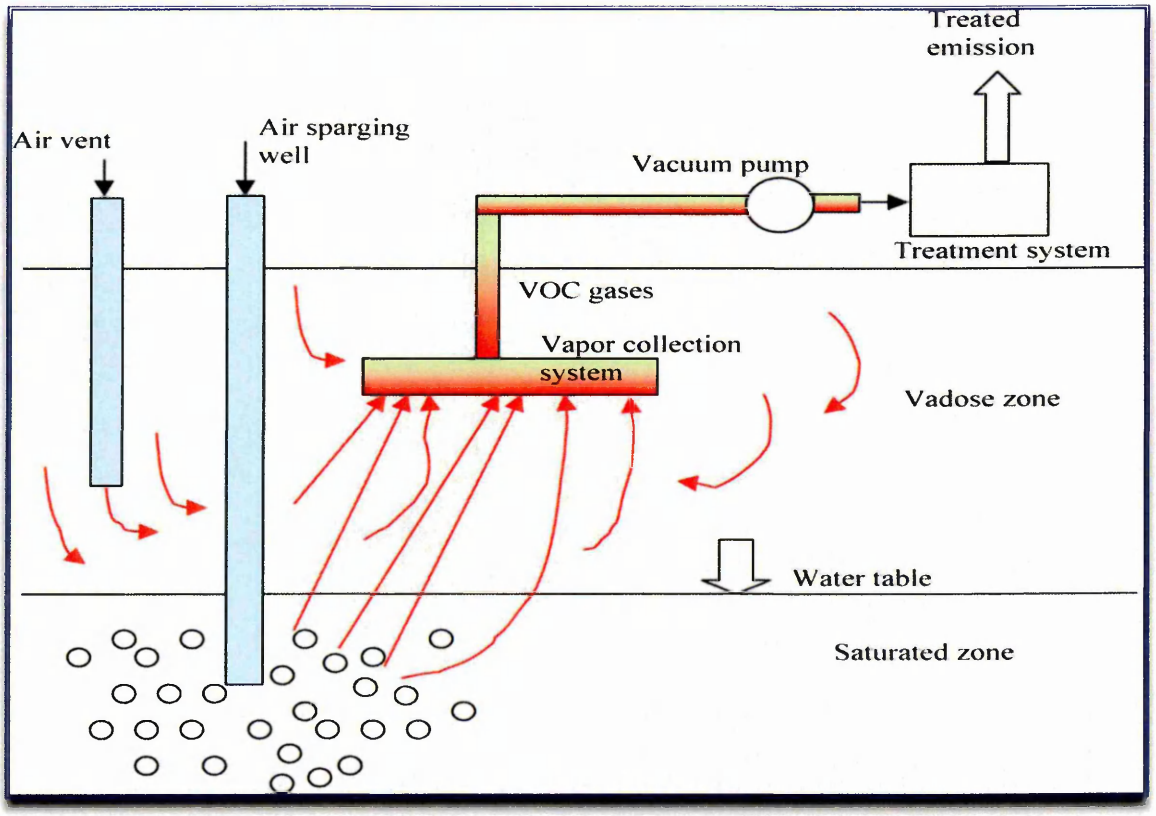
1.11.2 Air sparging

Air sparging is a physical and/or a microbiological degradation process and is used for the treatment of VOCs dissolved in water, sorbed to the saturated zone soils, and trapped in the pores of the saturated zone. Air sparging is an *in-situ* remediation method and the system consists of a network of air injection wells installed into the saturated zone and works by driving large quantities of air under pressure into the saturated zone such that hydrocarbons are transferred to the vapour phase. This technique enables an aerobic degradation of organics. The injection of air to the saturated zone serves two purposes (Halmemies at al., 2003, Khan et al., 2004 and Adams et al., 2011); first it drives the volatile organics into the unsaturated zone, from where they may be removed by vapour phase extraction system. Secondly it increases the microbial activity in the saturated zone to degrade the hydrocarbons. Air sparging and vapour phase extraction processes can be combined together as shown in Figure 1-11. Many studies have been performed to assess air sparging, involving either the evaluation of field studies, physical models, or mathematical models (Khan et al., 2004 and Adams et al., 2011).



Source: www.ctci.org.tw/public/Attachment/03310493371.pdf and Khan et al., 2004

Figure 1-10 Diagram of a typical bioventing system

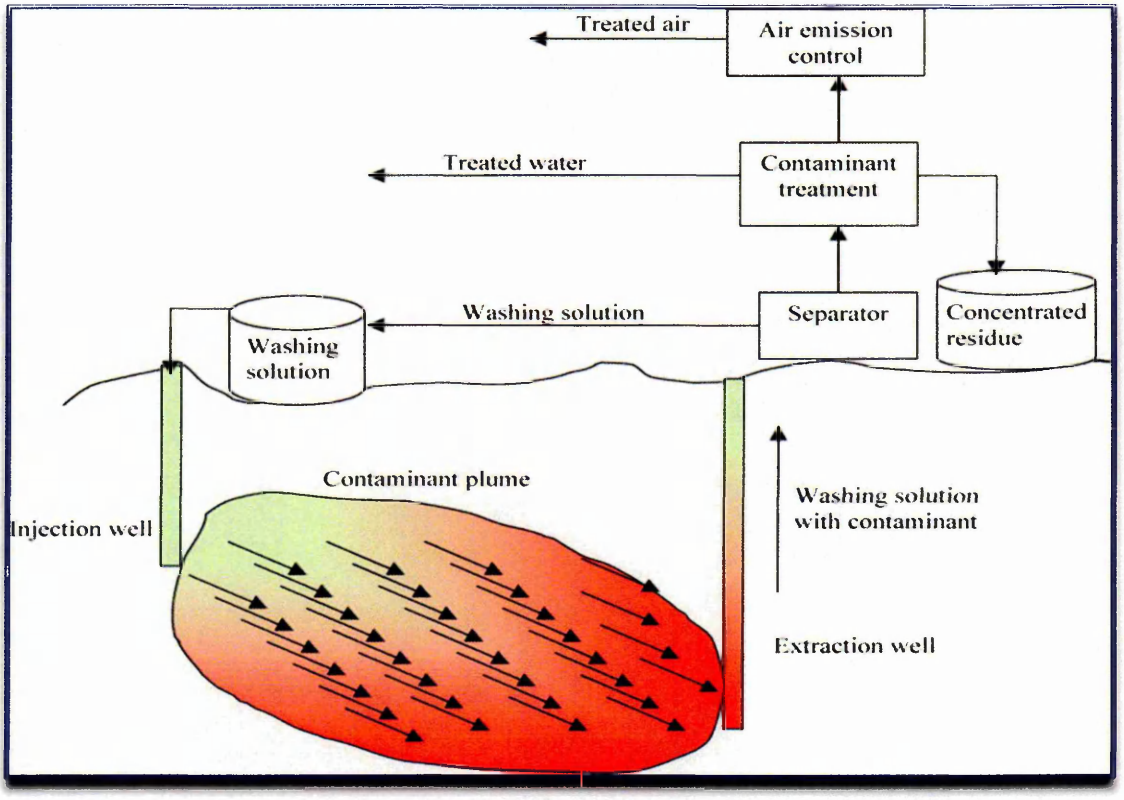


Source: Halmemies at al., 2003 and Khan et al., 2004.

Figure 1-11 A typical air sparging with soil vapor extraction system

1.11.3 Soil flushing

Soil flushing is *in-situ* remediation technology that operates through addition of solution (e.g. water or water plus a miscible organic solvent such as alcohol) through injection wells into either the vadose zone, saturated zone, or both to extract hydrocarbon contaminants from soil and transfer them to the groundwater, where they are subsequently captured and pumped to the surface through groundwater extraction wells as shown in Figure 1-12. The polluted water recovered is then treated to meet the discharge standards before recycle or re-use. Soil flushing is suitable for all types of soil contaminants and is generally used in conjunction with other treatment methods such as pump-and-treat, activated carbon and biodegradation. Since soil flushing is conducted *in-situ*, it reduces the need for excavation, handling, or transportation of hazardous substances (David 1996 and USEPA-forum 2006).



Source: Khan et al., 2004.

Figure 1-12 Diagram of a typical soil flushing system

1.11.4 Soil washing

Soil washing is a technique used to remove contaminants from soil by using liquids usually water or water combined with solvent and mechanical processes to scrub soils. The solvents are selected for their ability to remove contaminates from soil and for their effect to the environments and health. Soil washing can reduce the concentration of contaminants in soil by up to 90 %. Then after this treatment the soil is considered to be clean, non-toxic and can be re-used. The soil washing process separates fine soil (clay

and silt) from common soil (sand and gravel) (Khan et al., 2004). Soil washing is usually combined with other methods (such as incineration or bioremediation) to treat very fine soil (clay and silt) which still contains contaminants for safe disposal in accordance with regulations. Khan et al., 2004 has described different treatments methods available for dealing with soil and ground water contaminants with petroleum hydrocarbons. The author concludes that soil washing treatment technology is effective only for limited soil quantities but that it is efficient and fast although costly.

1.11.5 Solidification and stabilization (S/S)

Solidification and stabilization (S/S) are technologies where contaminants are physically bound or covered within a stabilized mass (solidification), or chemical reactions are encouraged between the stabilizing agent and contaminants to reduce their mobility (stabilization). They can be used to convert the hazardous compounds or contaminants into a less soluble, immobile and less toxic form, and are used to treat radioactive, hazardous, and mixed wastes (Khan et al., 2004 and Zain et al., 2010).

Zain et al., 2010 has applied solidification and stabilization technologies to reduce petroleum sludge in soil from Malaysia. The results showed success converted the organic contaminants were convereted by S/S system by adsorbing them onto the surface of cement used (i.e. significantly decreased the strength of the S/S products).

1.11.6 Asphalt batching

Asphalt batching is a stabilization/solidification method used to treat hydrocarbons in soils. The method involves excavation of the contaminated soils from the site, which then undergo an initial thermal treatment. This is then followed by incorporation of the treated soil into an aggregate for asphalt. During the process, heating of the mixture results in the volatilization of the more volatile hydrocarbon constituents. The remaining compounds are incorporated into an asphalt matrix after cooling, thereby limiting constituent migration. After it is given sufficient time to set and further treatment, the resulting solid asphalt now has the waste uniformly distributed throughout it and is impermeable to water (Khan et al., 2004)

1.11.7 Thermal desorption

Thermal desorption is a treatment technology where contaminated soil is excavated, screened, and heated to separate the contaminants from the soil. It involves heating soils in a chamber at certain temperatures, so that the contaminants with boiling points in this range will vaporize and separate from the soil. This includes organic contaminants and certain metals. A gas or vacuum system transports vaporized contaminants to an off gas treatment system. Thermal desorption aims to volatize

contaminants, while attempting not to oxidize them otherwise, thermal desorption would be simply a form of incineration. Thermal desorption can be divided into high and low temperature thermal desorption (Khan et al., 2004).

1.11.8 Aeration

This technology evaporates the volatile components of petroleum from the soil into the air. It is a well-developed process in which the area of contact between the water and the air is increased. The contaminated soil is spread thinly and tilled or turned to increase the rate of evaporation. The collected vapours also require further treatment. This simple methodology could have useful application in the summer treatment of waterlogged tundra soils. Aeration is often placed lower on the hierarchy of treatment technologies than those that destroy the contaminants. In groundwater, aeration brings about contact between the air and the water to promote biological degradation. It may be employed in activated sludge, rotating biological contactors, trickling filters and the biological lagoons (Khan et al., 2004). Many configurations may promote aeration including jets to blow air into the water or mechanical aeration devices that propel water droplets through the air.

Source: Images for land treatment, www.iwawaterwiki.org/xwiki/bin/view/.../LandTreatmentSystems.

Figure 1-13 Landfarming treatment method (KUWAIT)

1.11.9 Landfarming

Landfarming also known as land treatment or land application, is used for the treatment of oily petroleum industry wastes and is an above-ground remediation technology for

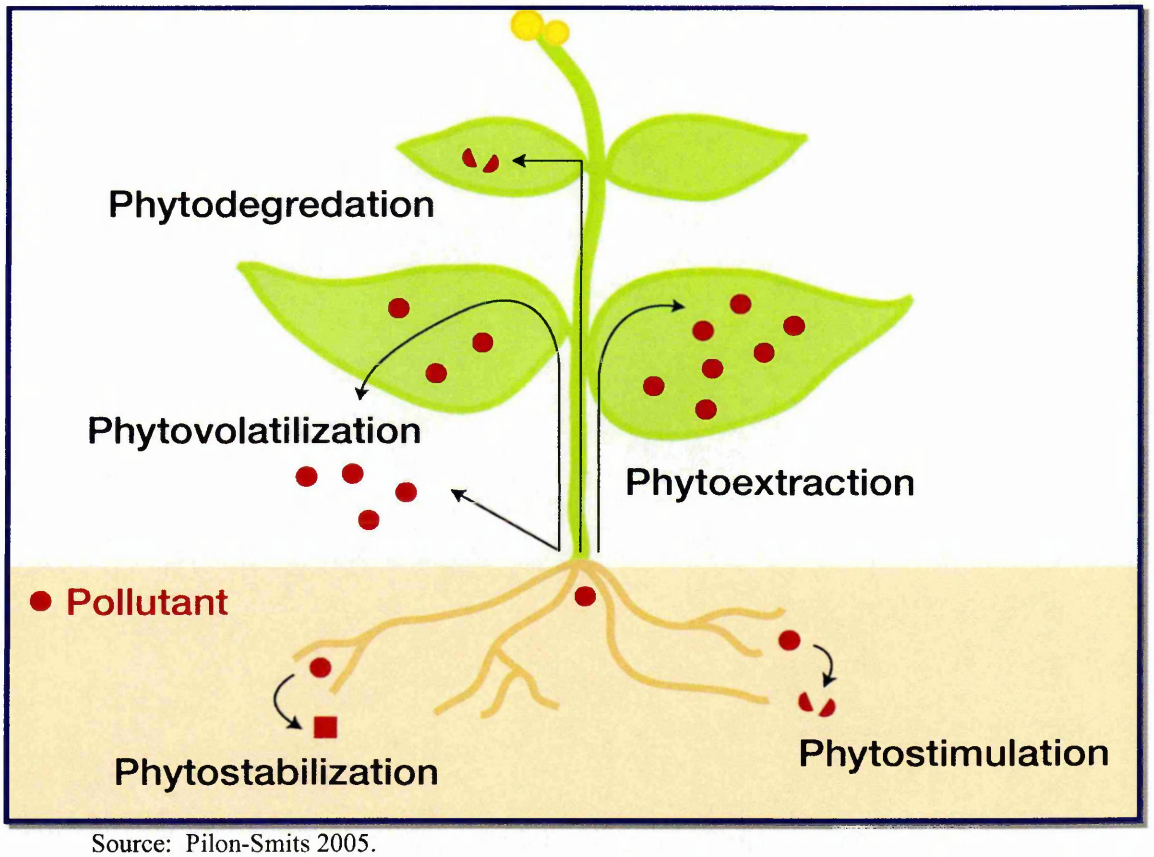
soils. Concentrations of petroleum pollutants are reduced through biodegradation under proper conditions by using microorganisms naturally present in soil. Landfarming usually involves the spreading of excavated contaminated soils in a thin layer (Maximum 1.5 metre) on the surface of a treatment area. Aerobic bacteria within the soils are enhanced through aeration, addition of nutrients minerals and the addition of water to the polluted soil as shown in Figure 1-13. Bacteria can be enhanced or added (enhancement of microbial activity) to this soil to improve success in breaking down hydrocarbon; this will help to transform contaminants into non-hazardous substances. The landfarming treatment has been applied commercially in large scale to remove the petroleum hydrocarbon from soil with relative success. It is used due to its simplicity and cost-effectiveness (Maila et al., 2004). Considerations for the application of landfarming include the site topography and hydrology, and the physical and chemical composition of the waste and resultant waste/soil mixture (E & P Forum 1993).

1.11.10 Phytoremediation

Phytoremediation describes the treatment of environmental problems through the use of plants that mitigate an environmental problem without the need to excavate the contaminated material and dispose of it elsewhere. Essentially, it is the use of green plants to clean-up contaminated soils, sediments, or water (i.e. uses plants to remove pollutants from the environment). In addition, the plants work with soil organisms to transform contaminants into harmless forms. Phytoremediation technologies have been used by many researchers and commercial operators to treat soil containing various environmental contaminants with different results. It is faster than natural remediation and has high public acceptance. For successful phytoremediation, both plants and microbes must survive and grow in crude oil contaminated soil (Cong et al., 2002, Dominguez-Rosado et al., 2004, Pilon-Smits 2005, Muratova et al., 2008, Basumatary et al., 2012 and Njoku et al., 2012). Figure 1-14 shows the phytoremediation mechanisms used to treat contaminated soil *in-situ* which are phytoextraction (the uptake of contaminant from the soil), rhizodegradation (the stimulation of microbial degradation through the activities of plants in the root zone), phytodegradation (use of plants to uptake, store and degrade contaminants within its tissue), phytovolatilization (use of plants to uptake contaminants from growth matrix and then transform volatilize contaminants into the atmosphere), and phytostabilization (uses plants to reduce the migration of contaminants through the soil medium).

Phytoremediation is applied for soil contaminated with low and moderate levels of contamination (i.e. high level of contamination could be toxic to plants).

Phytoremediation has been used to treat a wide range of contaminants such as heavy metals, VOCs, PAHs, petroleum hydrocarbons and radionuclides. It is less expensive than excavating processes, but may take a longer time than other technologies, to treat the site (Alkorta et al., 2001and Diab 2008[1and 2]). Atagana 2011 has studied the capability of Chromolaena odorata (L) plants to grow in different concentrations of crude oil contaminated soil in the presence of heavy metals and their capability to remediate the soil. This author found that the Chromolaena odorata (L) plants are able to grow in soil contaminated with 5 % crude oil in the presence of heavy metals the plant is able to cause the removal of both the contaminant oil by 82% and the present heavy metals by up to 65 %.



Source: Pilon-Smits 2005.

Figure 1-14 A typical phytoremediation mechanisms

1.11.11 Excavation

Excavation is the physical removal of impacted soils by conventional excavation techniques for subsequent disposal or treatment. Excavation and off site disposal is restricted to surface soils and can be expensive when indirect costs such as transportation and treatment and/or disposal are considered. Excavation and treatment of impacted soils reduces the hazardous nature on site, and can be accomplished either above ground or *in-situ*. On-site treatment of excavated material may include such strategies as land treatment or bioremediation, vapor extraction, thermal treatment,

washing, and chemical extraction, or some form of solidification/stabilization. Land spreading involves aeration by spreading of the soil in thin layers, applying moisture or nutrients if necessary, and allowing a combination of aeration and degradation of hydrocarbons to occur (Ulrich et al., 1996).

1.11.12 Natural attenuation

Natural attenuation is also known as passive remediation, *in-situ* bioremediation, native remediation, bio-attenuation, and intrinsic bioremediation. Natural attenuation is an *in-situ* treatment method that uses natural processes to reduce the concentration and amount of pollutants at contaminated sites by a variety of methods include physical (i.e. diffusion, sorption/desorption and volatilization), chemical (i.e. ultraviolet degradation and chemical immobilisation) and biological processes (i.e. aerobic and anaerobic biodegradation) without human intervention to reduce the concentration of contaminants from the environment. Natural attenuation processes are classified as destructive (i.e. they destroy the contaminant) or non-destructive (i.e. a reduction in contaminant concentrations occurs). Biodegradation (aerobic and anaerobic) is the most important destructive attenuation mechanism, while, sorption, dispersion, dilution from recharge, and volatilization are the most widely used non-destructive attenuation mechanisms. Natural attenuation is a proactive approach that focuses on the verification and monitoring of natural remediation processes rather than relying totally on engineered processes. Natural attenuation processes may reduce contaminant mass (through processes such as biodegradation); reduce concentrations (through simple dilution or dispersion); or bind contaminants to soil particles to prevent contaminant migration (adsorption) (Khan et al., 2004 and Roldan et al., 2010).

1.11.13 Bioremediation

The range of organic pollutants released into the environment will cause environmental and health-related problems that have detrimental effects on living beings. Conventional approaches (e.g. landfilling, recycling and incineration, etc.) to the remediation of contaminated sites are inefficient and costly and can also lead to the formation of toxic intermediates. Thus, biological treatment methods are preferable to conventional approaches because, in general, microorganisms degrade environmental pollutants without producing toxic intermediates. Biological treatment techniques are typically more economical than traditional methods and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies (Paul et al., 2005 and

Mercer et al., 2011).

By definition bioremediation is any process that uses microorganisms, fungi, green plants or their enzymes to degrade the environmental contaminants into less toxic forms and may be return the natural environment to its original condition. It is a process in which naturally occurring microorganisms (i.e. yeast, fungi, or bacteria) breakdown or degrades hazardous substances into less toxic or non-toxic substances. Bioremediation has the advantage that the toxic hydrocarbon compounds are destroyed rather than simply moved to another environment (air or water). The two main approaches to oil-spill bioremediation are: (a) bioaugmentation, in which oil-degrading bacteria or fungi are added to supplement the existing microbial population, and (b) biostimulation, in which nutrients or other growth-limiting co-substrates are added to stimulate the growth of indigenous oil degraders (Lee et al., 1997). Many organic contaminants such as petroleum can be biodegraded by microorganisms in the environment. Natural bacteria in the environment will use petroleum compounds as their primary sources of carbon and energy, thus biodegrading the compounds during the process (Mohamed et al., 2005, Ibekwe et al., 2006 and Mancera-Lopez et al., 2008). The microbes such as *Pseudomonas* sp., *Bacillus* sp., *Arthrobacter* sp., *Mycobacterium* sp., *Acinetobacter* sp., *Rhodococcus* sp., and *Corynebacterium* have been reported in petroleum pollution bioremediation (Atlas 1981, Song et al., 2006 and Onuoha et al., 2011). There are three processes by which microorganism aid in the breakdown of hydrocarbons: fermentation, aerobic respiration, and anaerobic respiration.

Bioremediation has its limitations like other technologies. The range of hydrocarbons that can undergo biodegradation is limited. It is generally accepted that low molecular weight hydrocarbons (i.e. saturated normal alkanes) are the most readily degraded in a mixture by microbes, but some contaminants, such as chlorinated organic or high molecular weights aromatic hydrocarbons, are resistant to microbial attack; they are degraded either slowly or not at all. Hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded (Atlas1981 and Zytner et al., 2006).

Many organic compounds present in soil may be biodegraded to CO_2 and water using natural biological processes. However, natural biodegradation of contaminants tends to be rate limited due to limitations on the biological processes. These limitations may be overcome by optimising the biological conditions. The most important factors for control of biological degradation of hydrocarbons are:

- Adequate supply of hydrocarbon degrading bacteria
- Availability of sufficient oxygen for cell metabolism
- Availability of nutrients necessary for optimum bacterial metabolism
- Moisture control
- Temperature
- pH
- Soil salinity (Li et al., 2000 and Tyagi et al., 2011)

Bioremediation has been considered a potentially useful tool in the cleaning of oil spills and the treatment of oil residues (Atlas et al., 1991 and Balba et al., 1998). However, the presence of hydrocarbon degrading strains in contaminated sites with petroleum hydrocarbon does not indicate that oil components will be metabolized. For example, nutrients necessary for cell growth may be present in limiting amounts. Biodegradation is usually carried out by the addition of nutrients and/or other growth-enhancing co-substrates to stimulate the increase of indigenous oil degrading microorganisms (Leahy et al., 1990, Alexander 1991, Bragg et al., 1994 and Mercer et al., 2011).

Bioremediation has been proposed as an alternative or complementary technique to treat contaminated soil with petroleum hydrocarbons. It mainly consists in optimizing the conditions for the development of naturally occurring hydrocarbon and chemical degraders by adding nutrients and maintaining aerobic conditions as necessary. Hydrocarbon biodegradation is affected by physical, chemical and biological properties of the environment. A number of researches have been carried out on the biodegradation of petroleum hydrocarbon contaminated soil, sludge, sediments and the marine environments by naturally-occurring microbes. The extent of soil hydrocarbon biodegradation may depend upon soil and crude oil types, concentration of total hydrocarbon, and nutrient growth stimulants based on optimum C: N: P ratios (Boyd et al., 1984, Zhang et al., 1992, Salanitro et al., 1997, Jorgensen et al., 2000, Sabate et al., 2004, Liu et al., 2009 and Tyagi et al., 2011).

A major aspect of engineered *in-situ* bioremediation is the addition of nutrients such as phosphorus and nitrogen, electron acceptors such as oxygen, and/or microorganisms to the contaminated site (Kim et al., 1998 and Tyagi et al., 2011). Microorganisms are often found in the soils which are able to degrade petroleum hydrocarbons, but bioaugmentation of the populations may increase the rate of bioremediation. Additionally, inorganic nutrient supplementation may speed up the process, because the presence of large quantities of oil results in a high C:N ratio which is adverse to microbial activity (Kim et al., 2005 and Tyagi et al., 2011). A proper site assessment

and investigation should be conducted for this purpose. Successful bioremediation programs require the application of strategies adapted to the specific environmental parameters of the contaminated site; treatability studies are a condition for success (Kim et al., 2005 and Tyagi et al., 2011).

1.12 Thesis objectives

The objective of this study is to provide a general assessment of any adverse environmental effects and propose remediation techniques for contaminated soil impacted by PW disposal at the Nasser oilfield owned by Sirte Oil Company. The site has a long history of petroleum production being in use since 1959. The study involves collecting several PW and soil samples from the disposal pit at the Nasser oilfield. It includes analysis of both PW and the impacted soil using conventional and recently developed analytical techniques to help to identify and assay the main species that cause pollution of the soil. It also includes an evaluation of the optimal conditions to effect bioremediation of contaminated soil. The requirements and the optimum crude oil degradation by bioaugmentation and biostimulation from crude oil contaminated soils will be compared. An initial study of the contamination of soil caused by PW and characterization of contaminants in soil round the oilfield will be carried out. This will inform the choice of suitable technology for remediation. This work will examine a potential route for the restoration of contaminated soil at oilfields. The evaluation of the optimal conditions to affect bioremediation of contaminated soil requires also investigation of the fate and transport of contaminants.

The final results of the study will hopefully be applicable to similar cases and suggest alternative environmental protection plans to the oil sector in Libya.

The outline of this thesis is as follows:

After the introduction chapter, Chapter Two is background, site history and field work. Chapter Three describes the methods and the results for physiochemical composition of PW and its impact on soil. Chapter Four describes the methods, instruments and setup for hydrocarbon analysis (TPH, BTEX and PAH) in soil and PW. Chapter Five describes the use of ESI/MS/MS and LC/ESI/MS techniques to Analysis of oilfield chemicals in soil and PW. Chapter Six is devoted to detailed results on enhanced biodegradation of petroleum hydrocarbons in contaminated soil. Finally, in Chapter Seven, conclusions are drawn and suggestions for future work are presented.

Chapter Two
Background, Site history and Field work

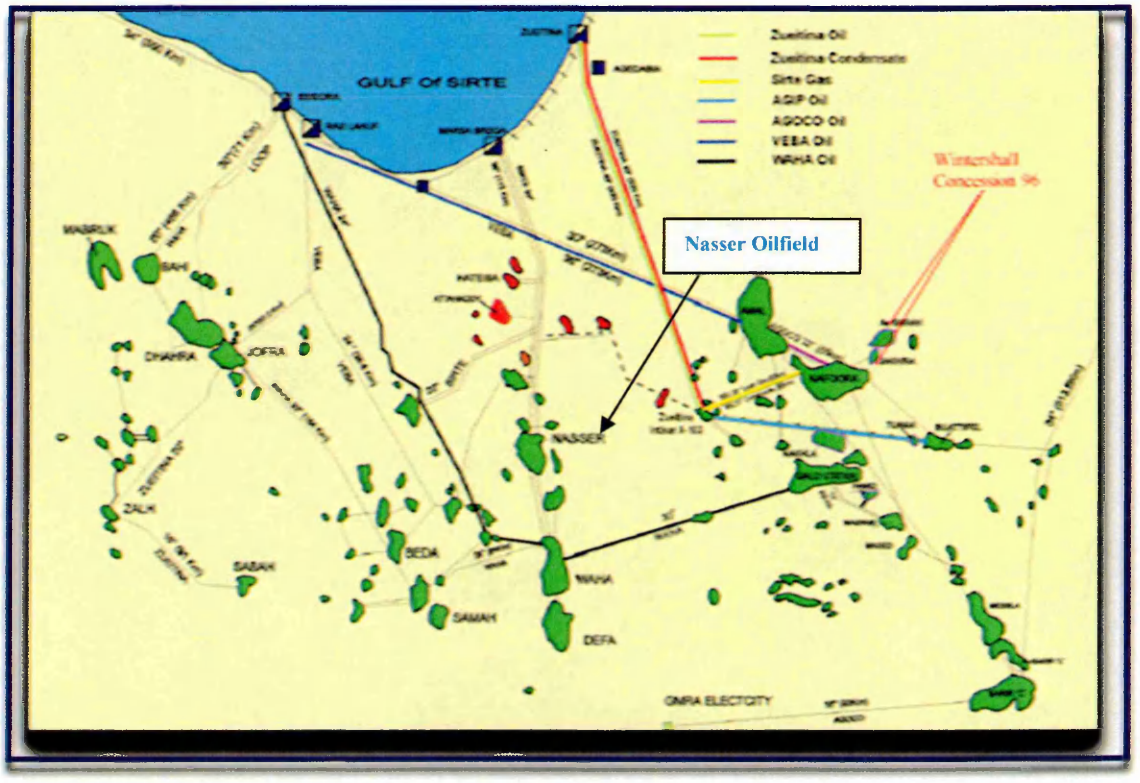
2.0 Background and Site history

2.1 Purpose

Produced water (PW) volume generation and management in the Libyan oilfield are not well characterized at a national level. The purpose of this study is to improve understanding of PW by providing complete detailed information on the volume and compositions of PW generated in the studied oilfield and the ways in which PW is disposed or reused. Furthermore it aims to provide a general assessment of any adverse environmental effects on the ecosystem and propose remediation techniques for contaminated soil impacted by PW disposal.

2.2 Site location

Sirte Oil Company (SOC) for production, manufacturing of oil and gas is one of the Libyan national companies operating under the National Oil Corporation (NOC) of Libya. The company headquarters and complexes are located in Marsa El Brega, which is 800 km east of Tripoli. The Nasser oilfield (also known as the Zelten oilfield) which has been chosen as the study area is owned by SOC and is located in the north central part of Libya which is 180 km south of Marsa El Brega in concession 6 at 28° 57' 33".8 N and 19°41' 37".9 E as is shown in Figure 2-1.



Source: Libyan Petroleum Institute

Figure 2-1 Location of the Nasser oilfield

2.3 Site history

SOC for production, manufacturing of oil and gas was the first company to discover oil in commercial quantities in the Nasser oilfield. In fact the first oil well was drilled at the Nasser oilfield in 1956, and placed into production in 1961; additional wells were drilled at the field after that subsequently to make a total of 229 wells on production at the Nasser oilfield. The main stream coming up from the reservoir consists of oil, gas and PW. The producer has to separate these three components through a separation process, which use separator equipment. In order to improve the separation of the components of the mixture one from the other, it is advantageous to employ treatment chemicals, where a predetermined flow rate of treatment chemical is injected into the water, oil and gas fluids at the inlet. At the Nasser oilfield and due to the high percentage of water generated daily in the field, the Gas Oil Separation Plant (GOSP) is employed to separate the gas (only) from the mixture., thereafter the oil and water mixture is pumped to the tank section, and by the use of chemical treatment (Demulsifiers), the oil is separated from the water at a certain resident time as shows in Figure 2-2. Then downstream PW is drained and directly pumped throughout pipeline and discharged into the surface into the open evaporation pits as shown in Figure 2-3.

Figure 2-2 A mixture of crude oil and produced water before and after separation

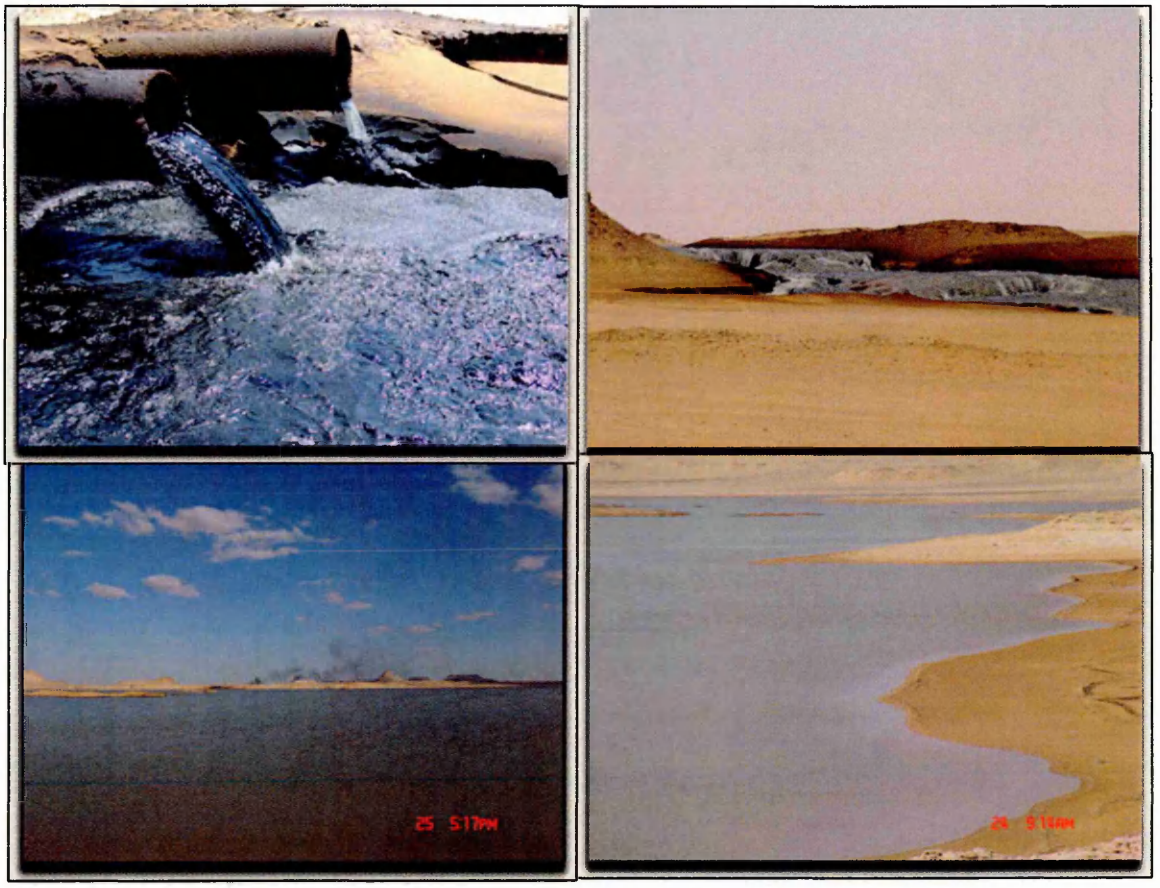


Figure 2-3 shows the produced water discharge point (DP) from facilities and the side view of produced water, Nasser oilfield, Libya

2.4 Problem description

The separation, handling and disposal of PW represent the single largest waste stream challenge facing the oil and gas production industry. The disposal of PW and other aqueous effluents arising from oil production has always been a problem in remote locations, whether in the desert or in the jungle. In recent years, the deserts have come to be recognized as an important environment with governments now imposing regulations to control oilfield pollution of the desert. In many instances, PW is also being seen as an important water source which should not simply be evaporated or drained away. The evaporation pond is becoming ever less acceptable, requiring the adoption of other strategies to deal with PW, because of environmental protection concerns increased at the national and international level. The NOC has been intensifying the pressure on operating companies to make best use of all their assets. The two main concerns of NOC are to increase production and to reduce the impact of oilfield operations on the environment. One element of the assets that is now taking a higher profile is PW, due to its potential value and environmental impact. In the process of extracting oil and gas in SOC, more than 315000 barrels of PW are generated daily in the Nasser oilfield on 2008 and it is directly discharged into the surface onto an open

evaporation pit which is cover about 11 Km^2. It is felt that much of these oilfield-produced waters can be treated and reused. According to field investigations during the site visit, the site is highly polluted by oily PW disposal as shows in Figure 2-4. Many activities have been carried out at the site during the field work for this study to fulfill the proposed targets.

Figure 2-4 Accumulated of crude oil and salt in the soil at the bank pit of the Nasser oilfield, Libya

2-5 Site topography

The topography of the study area was generated by taking fifteen bench marks which are selected from Geological map of Libya- Bi'r Zaltan Sheet-prepared by Industrial Research Centre (IRC) (Domaci and Maqtof 1985). The coordinates of each point and their elevation are used as input data for Surfer8 mapping software, to make two and three dimensional topographical maps. The topography of the study area varies in elevation between 120 and 240 meters above the sea level. The topographical map shows that the pit under study is bounded by two relatively high areas to the north (240 meters above the sea level) and to the south (180 -200 meters above sea level) of the pit. The north eastern and the south western sides of the pit are relatively lower areas (120 and 140 meters above the sea level) as shown in Figures 2-5A, 2-5B & 2-6.

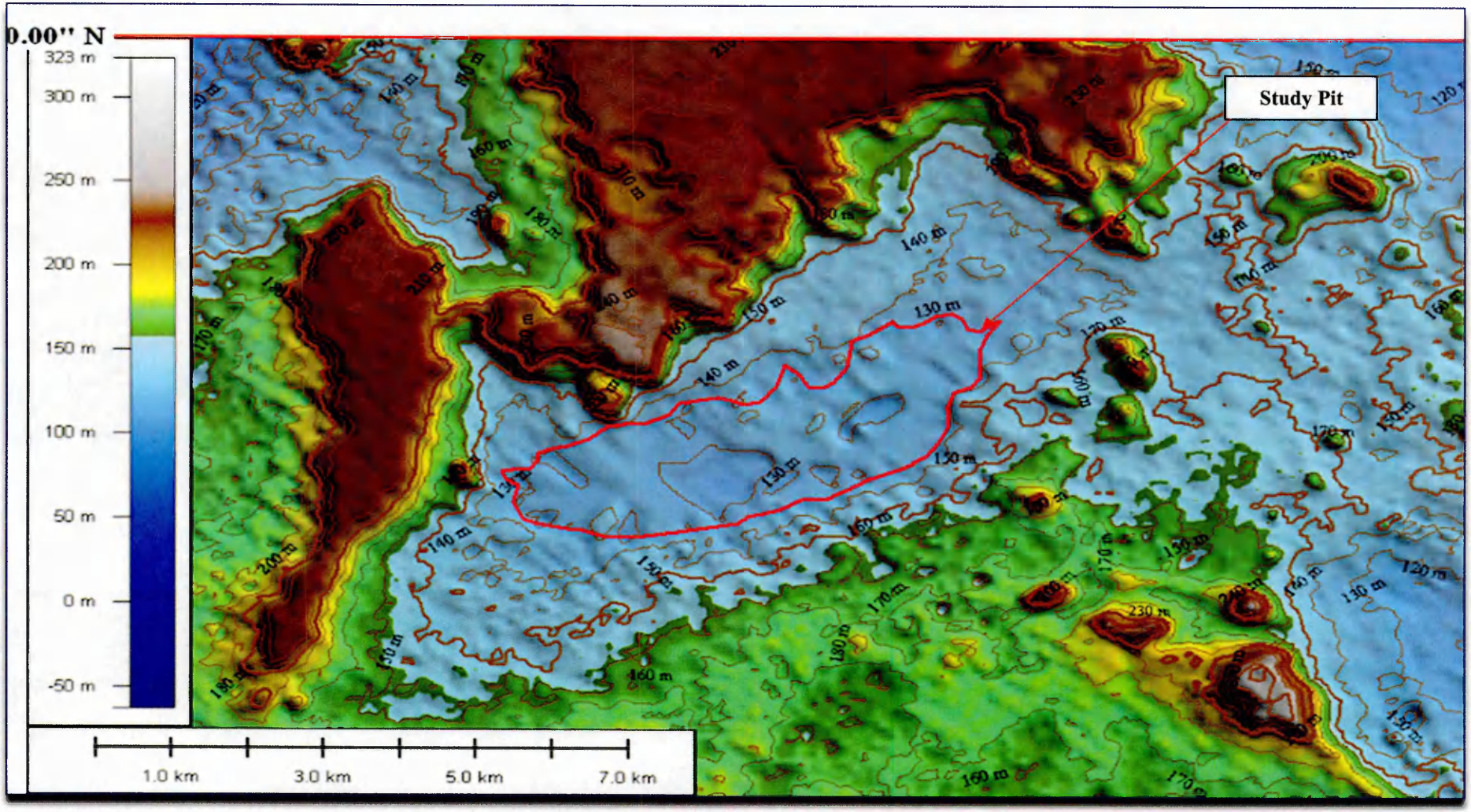


Figure 2-5A Topographical maps of the study site and the surrounding area

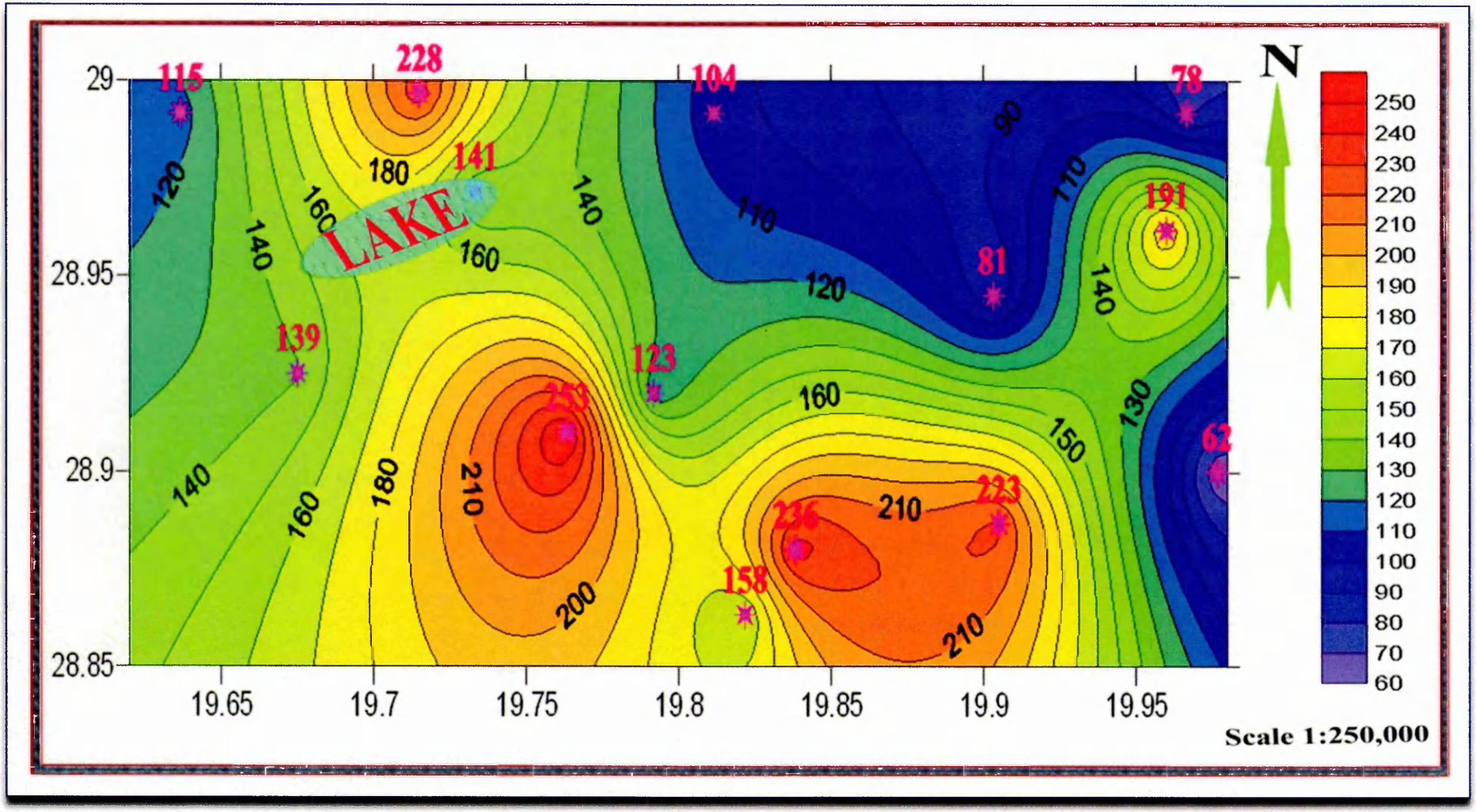


Figure 2-5B Topographical maps of the study site and the surrounding area

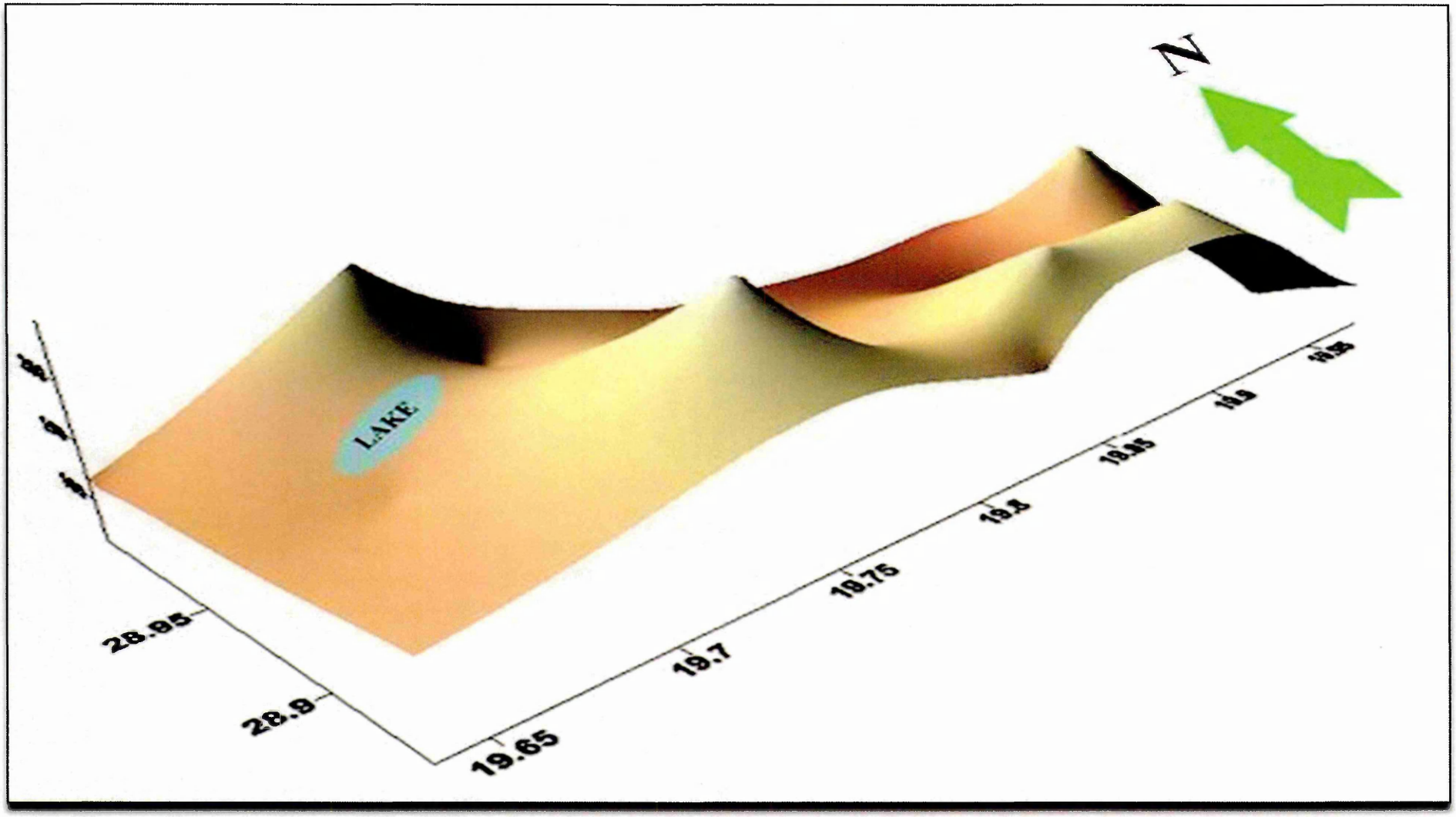


Figure 2-6 Three dimensions topographical map of the study area

2.6 Site hydrology

According to the available data collected from the Sirte Oil Company (SOC), Libyan Petroleum Institute (LPI) and General Water Authority (GWA), the aquifer in the study area is a confined aquifer in which the water rises to a certain level due to the confining pressure and is capped by impermeable layers (clay), the hydraulic conductivity was 4.3 $\times 10^{-3}$ m^2/s, the storage coefficient 5 $\times 10^{-4}$ and water levels of the aquifer ranges from 65 to 80 meters below the ground level.

2.7 Site geology

According to the geological study carried out by LPI and the information collected from the geological map of Libya- Bi'r Zaltan Sheet-prepared by the IRC. The study area is made from Maradah Formation which is widespread all over the land of the Bi'r Zaltan map sheet, except in its southwest part (Domaci and Maqtof 1985).

Maradah Formation is made up from two members; the lower part is Qrarat Jahannam Member (consists mostly of clastic sediments of estuarine and shallow-sea origin), the upper part is Ar Rahlah member (consists of facies of carbonate rocks and terrigenous clastics) (Domaci and Maqtof 1985).

The sedimentological study carried out by the LPI indicated that the lithologic units beneath the pit consist mainly of sandy sediments interbedded at different depths with claystone. The rock outcrops in the area around the disposal pit suggests that these sediments could represent the upper part of Qrarat Jahannam Member. The study identified two different types of the sandy sediments, sandy limestone and calcareous sand. The geological study shows that the site consists mainly of sandy sediments interbedded with claystone (Domaci and Maqtof 1985).

2.8 Site selection and preparation

The PW pit at the Nasser oilfield, Libya was selected for this study. Contaminated water has penetrated the soil to varying depths, depending upon the nature of the underlying soil. In June 2008 a site investigation of the produced water pit at the Nasser oilfield, Libya was carried out. The purpose of the site investigation was to obtain an overall picture of the contamination present on the site by visual observation, photographic documentation, sampling and chemical analysis. These investigations were also required to provide an understanding of the site geological, chemical and microbial characteristics. The site investigation carried out was used as a reconnaissance survey to obtain rough assessment of the extent and distribution of the contaminants present on the PW pit. The distribution of the contamination into the soil of the selected area was investigated by collecting surface soil samples (0-20cm below the surface).

2.9 Site climate

The Nasser oilfield has an extremely arid climate with small annual rainfall, precipitation, when it comes is usually in the winter months and is less than 50 mm. Relative humidity at the Nasser oilfield ranges from 65% in winter months down to less than 35% in summer session. Daily temperature varies on a seasonal basis, the mean temperature at the Nasser oilfield 24°C. The coldest average temperature was -2°C in December to January (during night). The hottest temperature was recorded between June to August with an average of 46°C. The prevailing wind direction is from the north, periodical winds bring sand and dust storms. The secondary winds at the Nasser oilfield during December to March were from the west and south and during October to November are from the south. The maximum wind speeds are 15 miles/hour during winter and spring and the lowest wind speed value are 5 miles/hour during summer.

2.10 Scope of work and objectives

The objective of this study is to provide a general assessment of any adverse environmental effects and propose remediation techniques for contaminated soil impacted by produced water disposal by:

1. Physically and chemically characterizing impacted and unimpacted soil.
2. Identify the type and extent of contamination
3. Identifying environmental hazards and risks associated with the disposal of produced water.
4. Characterization of the discharge – quality and quantity of the effluent.
5. Characterization of parameters relevant for microbial growth (nutrients, pH, T, EC and TPH).
6. Biodegradation experiments in soil under a range of conditions.
7. A high level evaluation of the environmental risks related to the disposal of produced water from the Nasser Field.

2.11 Field work

The field work was conducted in Nasser oilfield between 26/06/2008 to 06/07/2008. Many activities were carried out at the site to fulfill the proposed target. The activities included; drawing a map which shows the study area producing a sampling plan, carrying out a geological, hydrogeological survey, and sample collections (produced water, soil, crude oil and oilfield chemicals (OFCs) samples).

2.11.1 Site mapping

A survey had been done to map site boundaries, buildings, disposal pit, gas oil separation plant (GOSP) and storage tanks (A, B and C), as shown in Figure 2-7.

2.11.2 Disposal pit survey [Size, expended, depth, and quantity]

A survey of the pit was carried out in order to estimate the variations in depth, size and to collect PW samples from different points at the disposal pit.

2.11.2.1 Pit expansion and size

The historical expansion of the Nasser oilfield has been studied to clarify the magnitude of the problem associated with disposal of PW and its effects on the surrounding environment. Satellite images taken of the area during the time period from 1972 up to 2006 have been examined. The size and expansion of the pit has been identified as follows

Satellite image taken by Landsat MSS in 1972 is shown in Figure 2-8, for the study area. The image shows that in 1972 there were three pits located in the area. The pit under investigation is in the top north-west of the image. Pits are visible in the images as black pools. The size of the pit in 1972 was 1.8 km^2 according to the measurements made from the Landsat MSS satellite image.

Photography was carried out in 1978 of the study area. This image shows that the pit has extended to the southwest and produced another pit separated by a barrier to give total size of 6 km^2 as can be seen in the image of Figure 2-9.

Figure 2-10 shows the satellite image taken by SPOT XS in 1988 for the study area. The image revealed that the pits shown in previous Figure 2-9, have become one lake and the barrier extremely reduced. This is because of the amount of the water that was produced from the field and pumped into the disposal pit. According to the images suppliers (LPI) measurements of the size of the pit in 1988 was 8 km^2.

According to the image in Figure 2-10, the pit located in the east of the field camp seems to be start drying and disappearing, while the one that located in the southern part near to the field camp has been partially observed by smoke.

Figure 2-11 shows a satellite image taken by Landsat 7 ETM for the study in 2004. Comparing this image with previous image in Figure 2-10, the expansion of the pit into the west can be observed. According to the images suppliers (LPI) measurements the size of the pit in 2004 was 10 km^2. The other pit located in the eastern part of the camp field seems to be almost completely dried up and has disappeared except for a small part in the western boundary of the pit that is still filled with water.

Figure 2-12 shows the satellite image taken by Landsat 7 ETM for the study area in 2006. It is clear from the enlargement of the portion of the image containing the main PW pit under the study that there are no significant differences in the shape and size compared to 2004 as shown in Figure 2-11. The pit expansion was more to the western part and according to the images suppliers (LPI) measurements; the size of the pit in 2006 was about 10.7 km^2.

Figure 2-13 shows a map combination of the satellite images from 1972 to 2006 showing the size changes with times. From the figure it is clear that the pit has expanded mostly north-east to south-west and the biggest change was between 1972 and 1978 when the pit increased dramatically from about 1.8 km^2 to 6 km^2. This may be as a result of this coinciding with the peak of field production during that period.

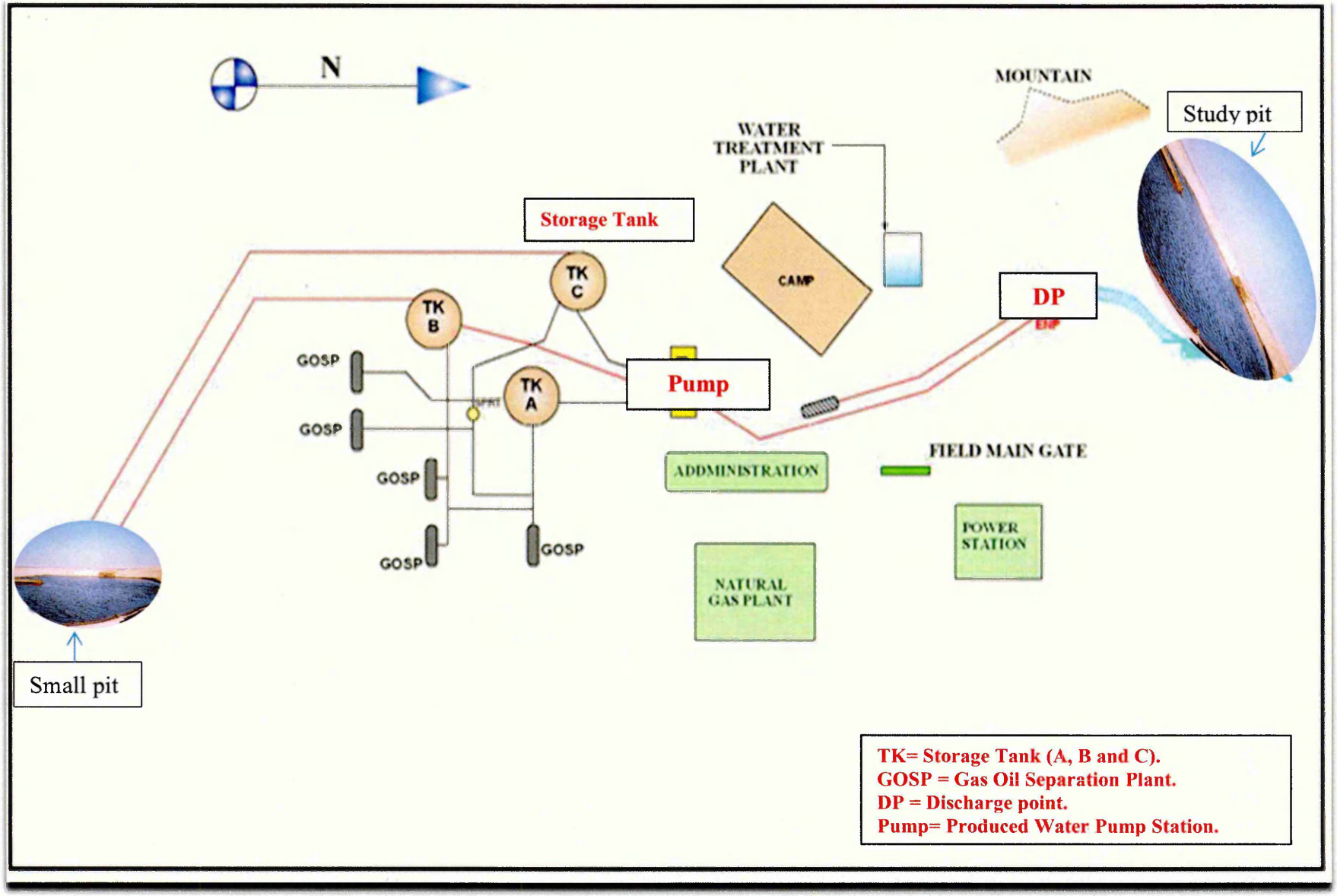


Figure 2-7 Schemes for the Nasser oilfield, Libya

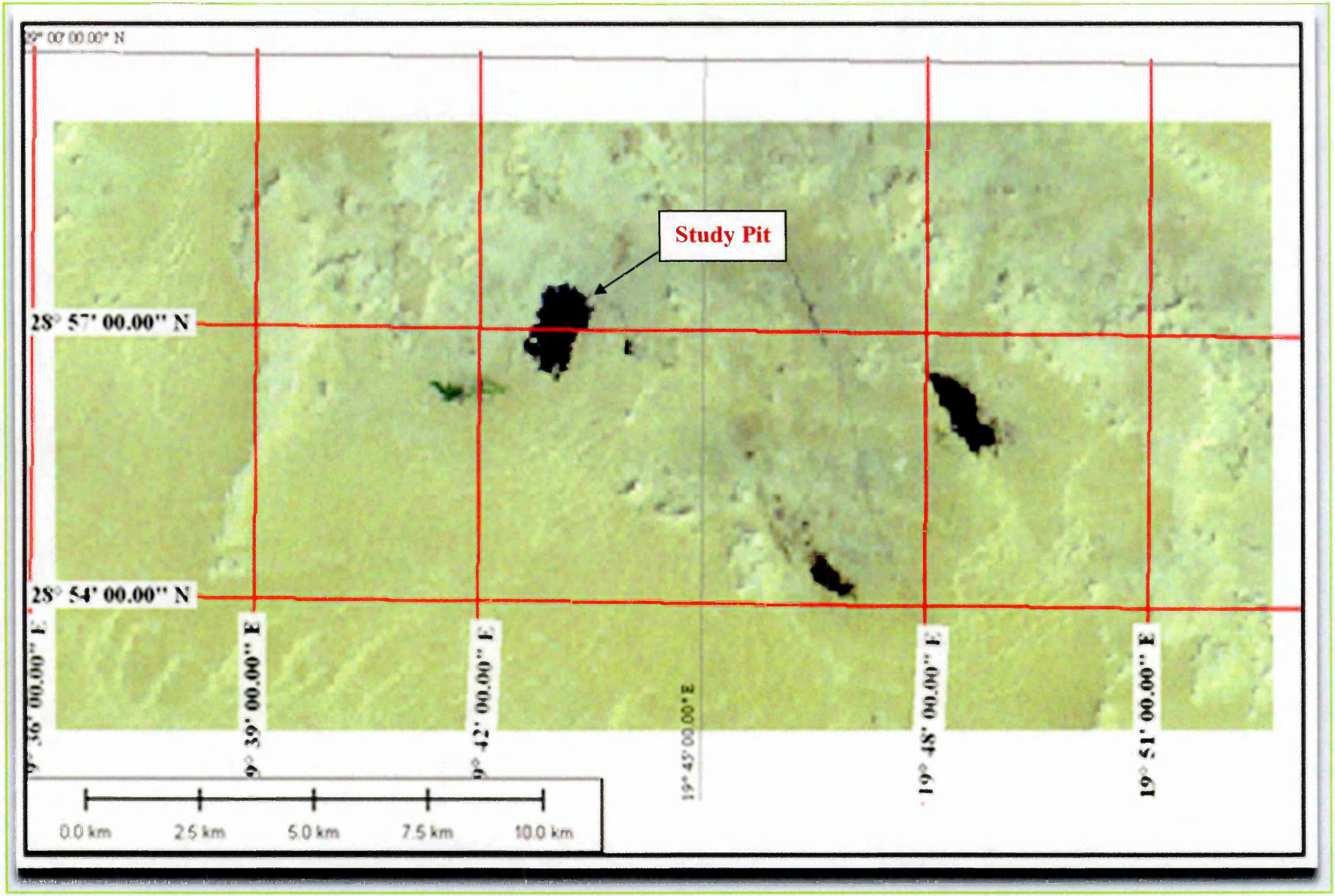


Figure 2-8 Satellite image (Landsat MSS) for the pit in 1972

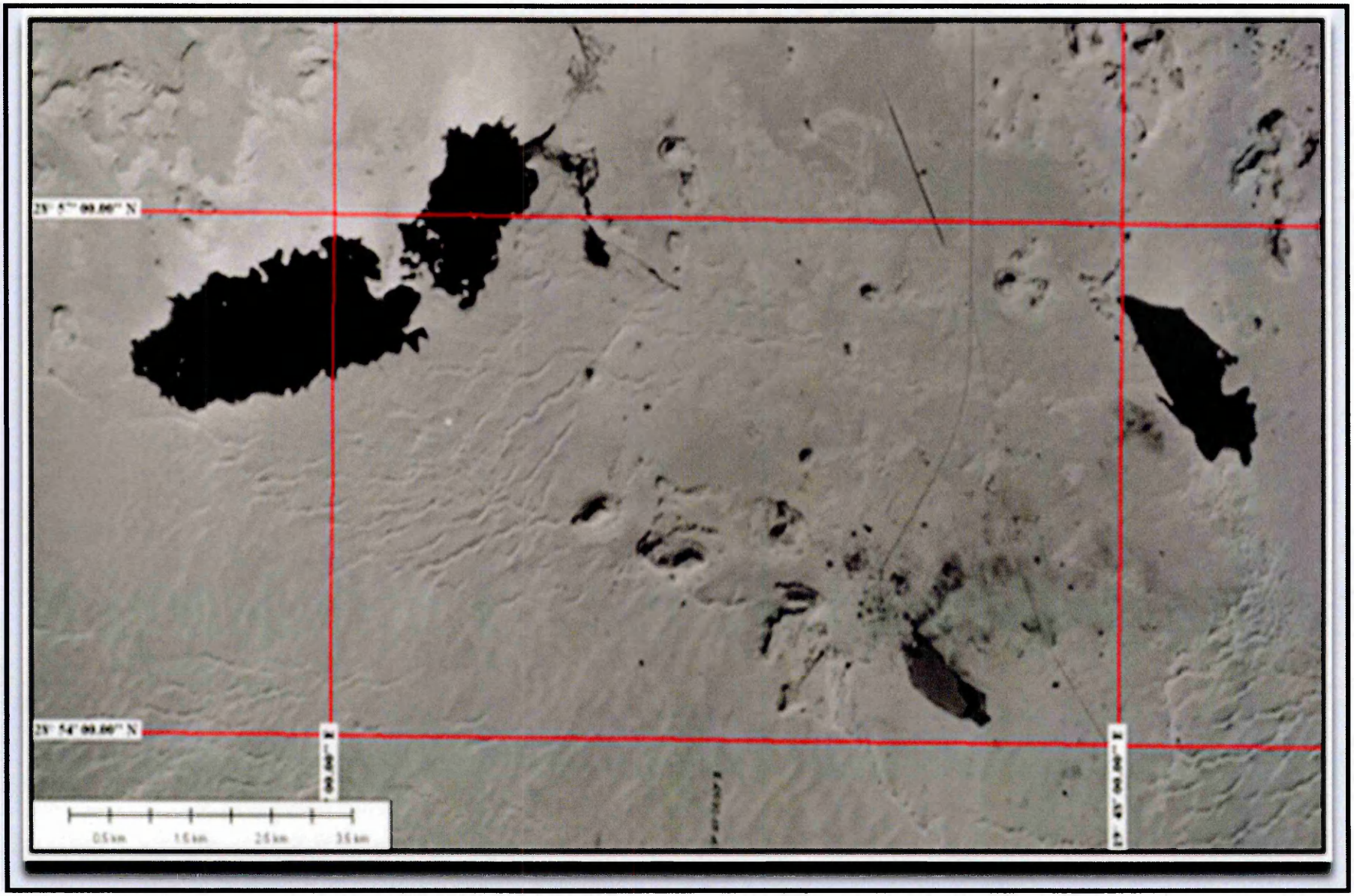


Figure 2-9 Arial photography of the pit area in 1978

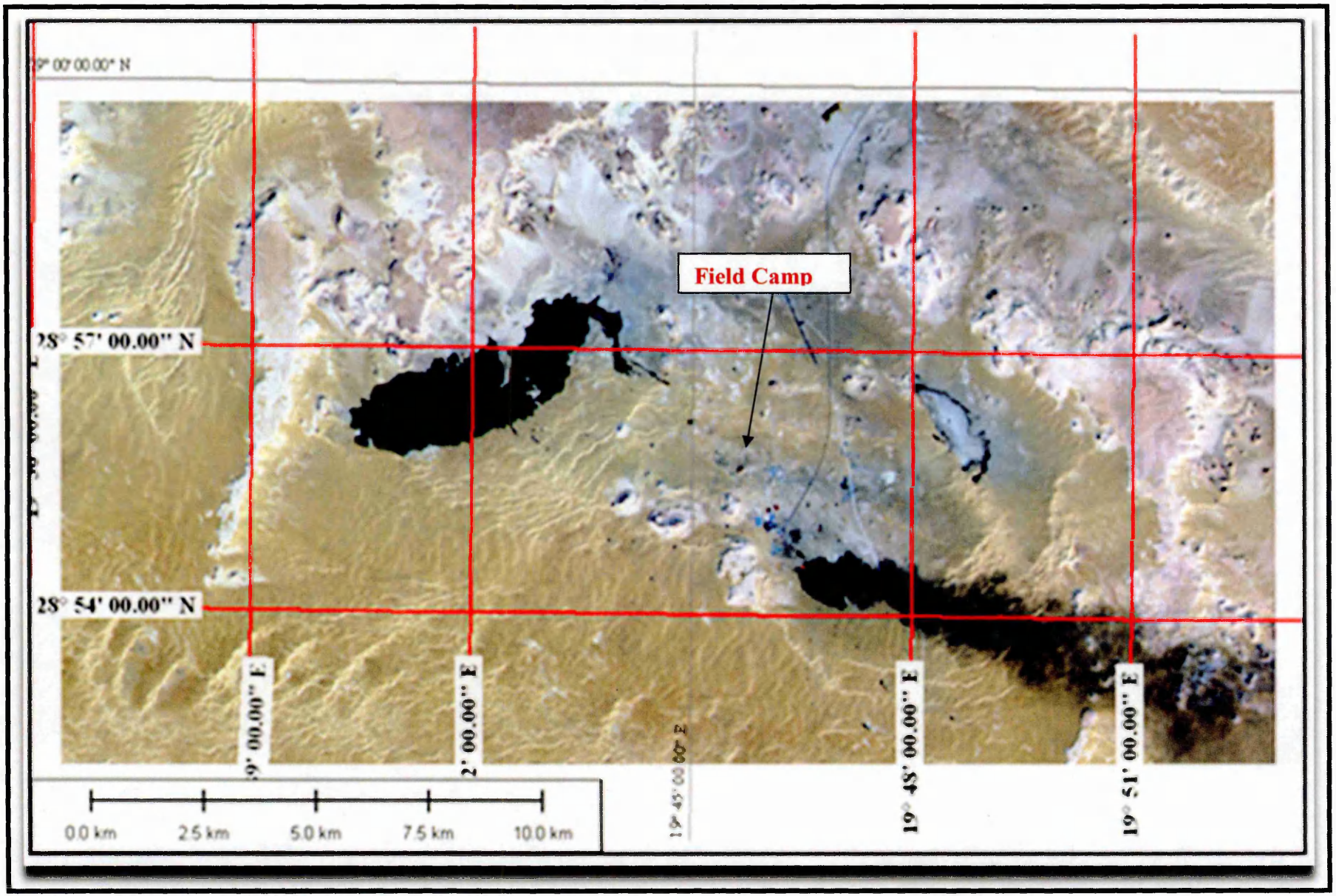


Figure 2-10 Satellite image (SPOT XS) of the pit area in 1988

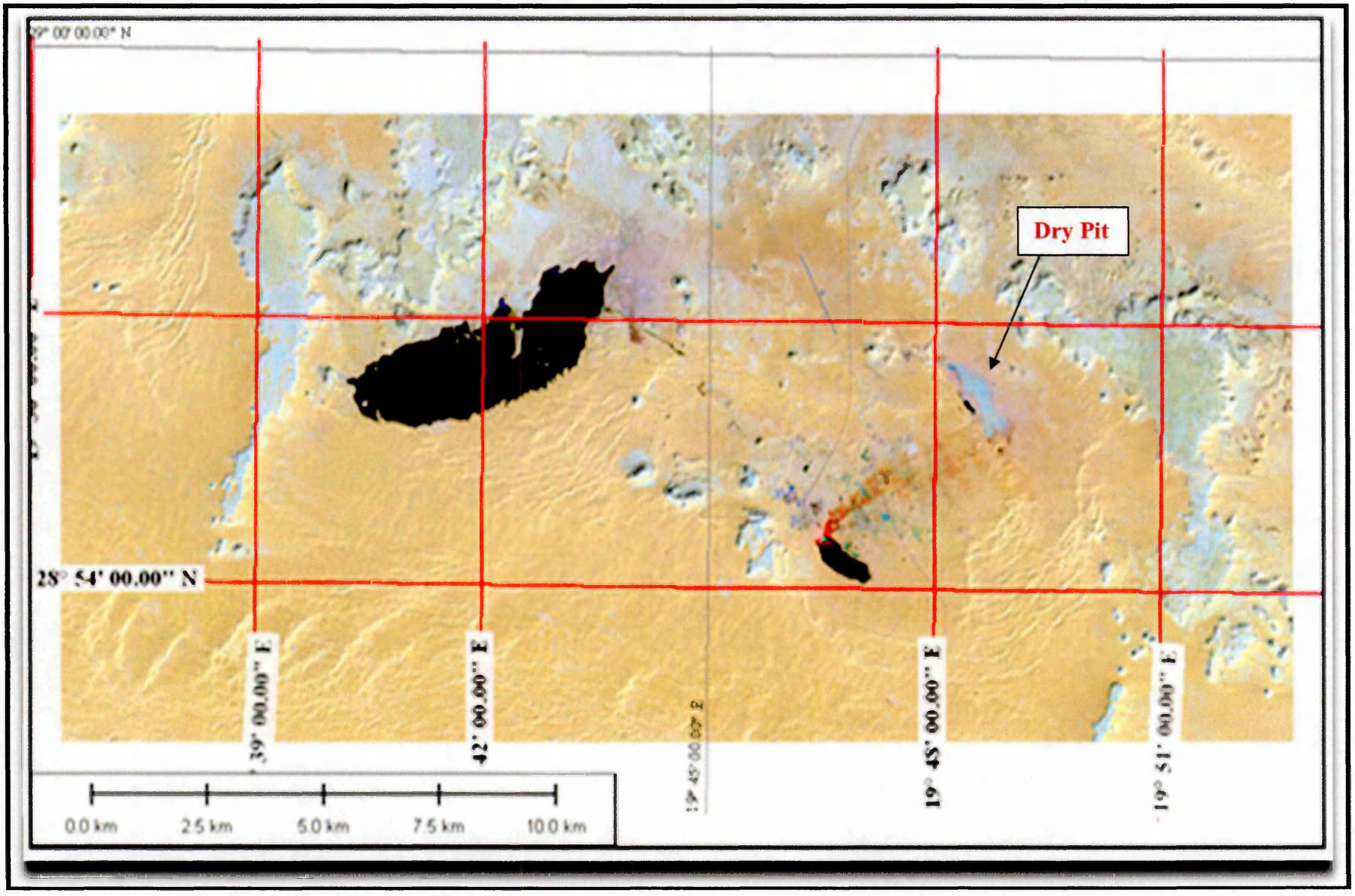


Figure 2-11 Satellite image (Landsat ETM 2004) of the pit area

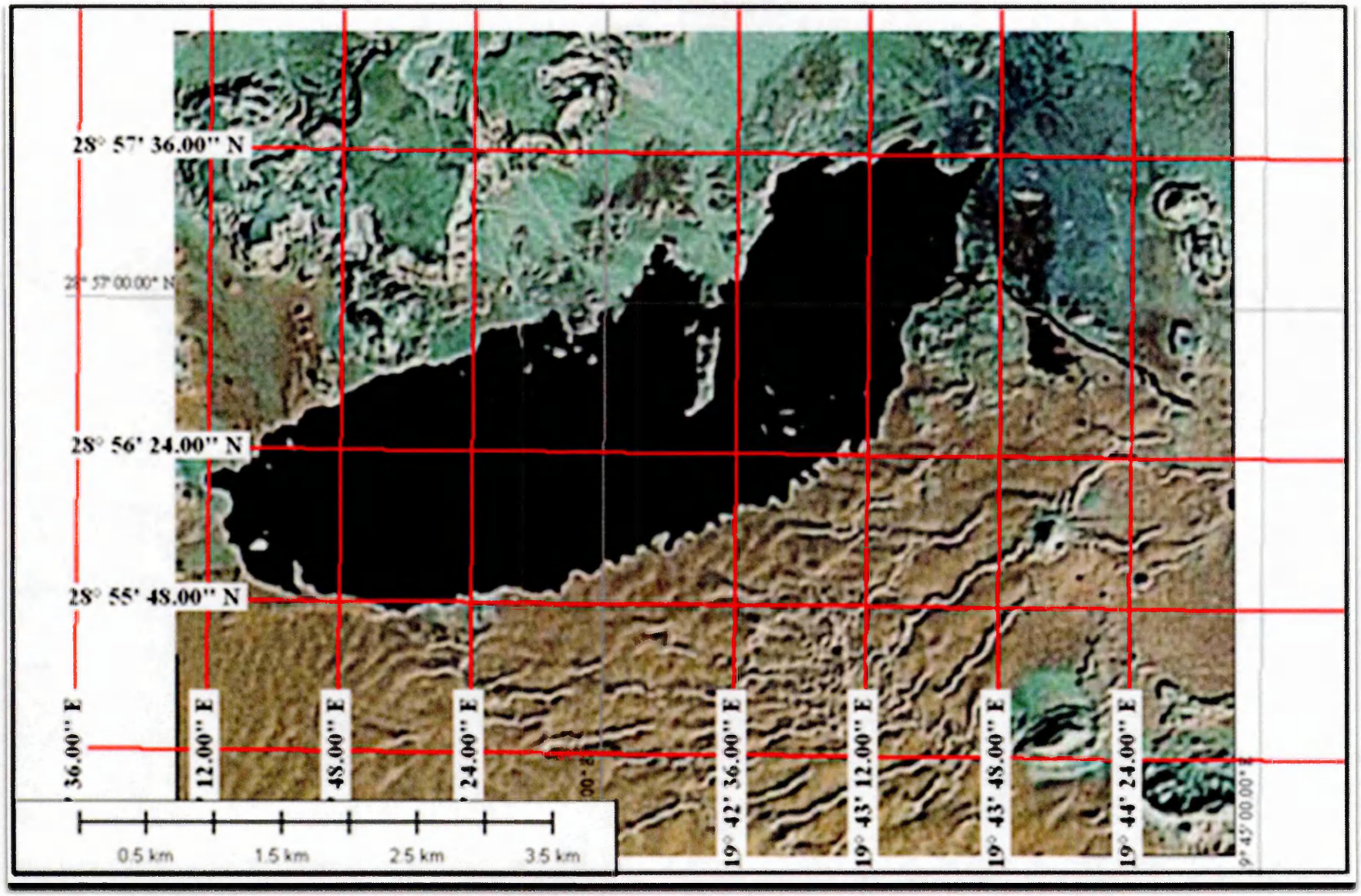


Figure 2-12 Satellite image (Landsat ETM 2006) of the pit area

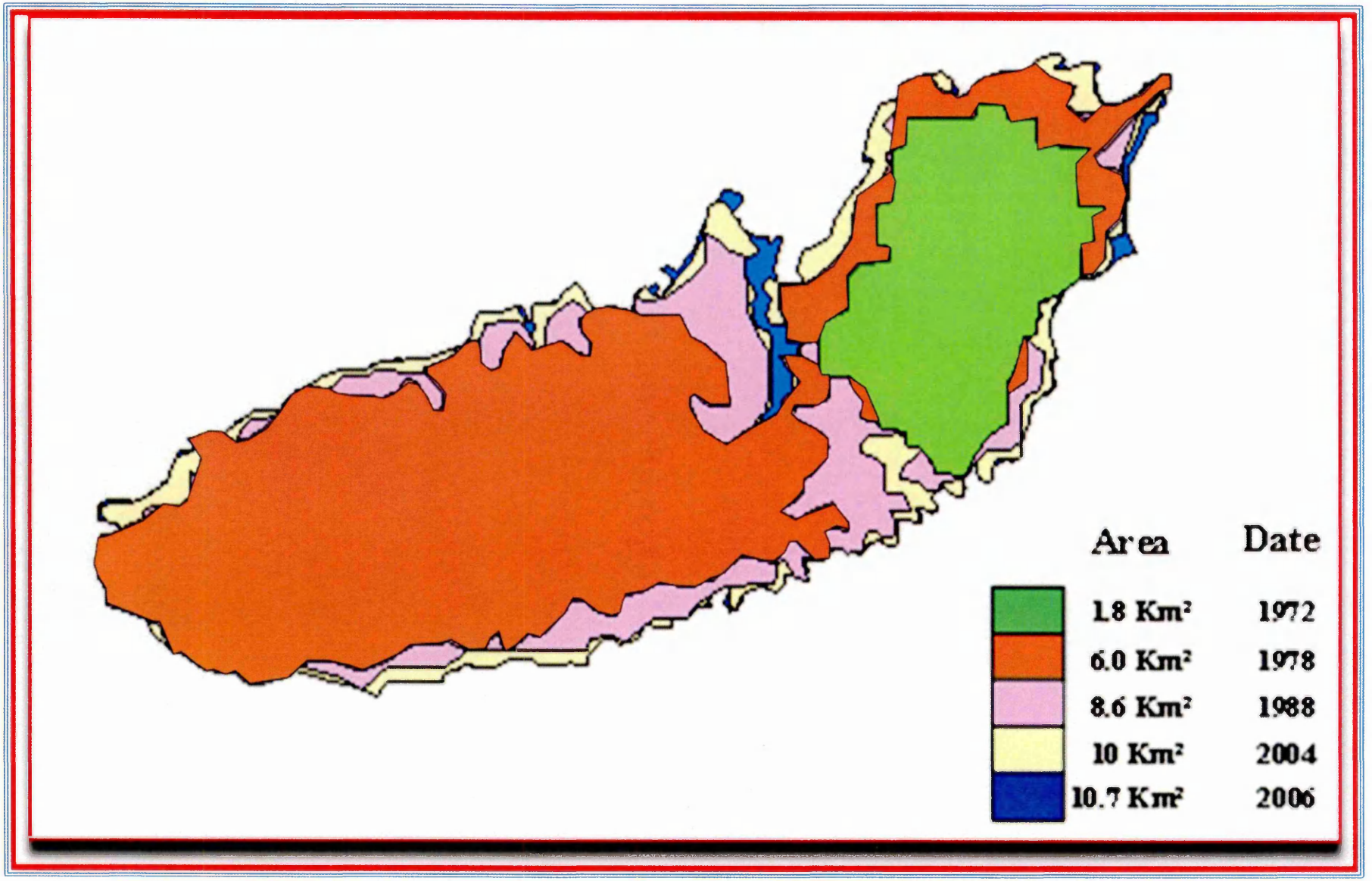


Figure 2-13 Status of the pit at the Nasser oilfield from 1972 to 2006

2.11.2.2 Pit depth

The depths of the pit were measured using a boat, weight, and meter tape at some random points into the pit and the depth of each point was recorded and the coordinates were taken using Global Position System (GPS), then satellite image processing has been used in combination with field measurements. The measurement show that the pit has different depths where the largest area of the lake about 4.2 km^2 with a depth from 5-8 meters while the shallowest area is about 0.6 km^2 with a depth less than 2 meters and the whole size of the pit covers an area of more than 10.7 km^2. Figure 2-14 is a classified map of the main pit under study which shows the distribution of the depths with their sizes based on field measurement and satellite image processing.

2.11.2.3 Produced water volume

Large volumes of PW from the Nasser Oilfield are produced and are discharged into the open environment and cause an environmental problem to the ecosystem. The water production at the Nasser Oilfield started in 1969s, the water production increased year by year. A total of 122 million cubic meters of PW has been produced from the Nasser oilfield since 1999 to 2008 as shown in Table 2-1, and a dramatic increase was noticed in the 2003 as shown in Table 2-1 and Figure 2-15. This increase resulted because the volume of PW relative to petroleum increases with time, normally reaching 90 % of total fluids during the later stages of the field production.

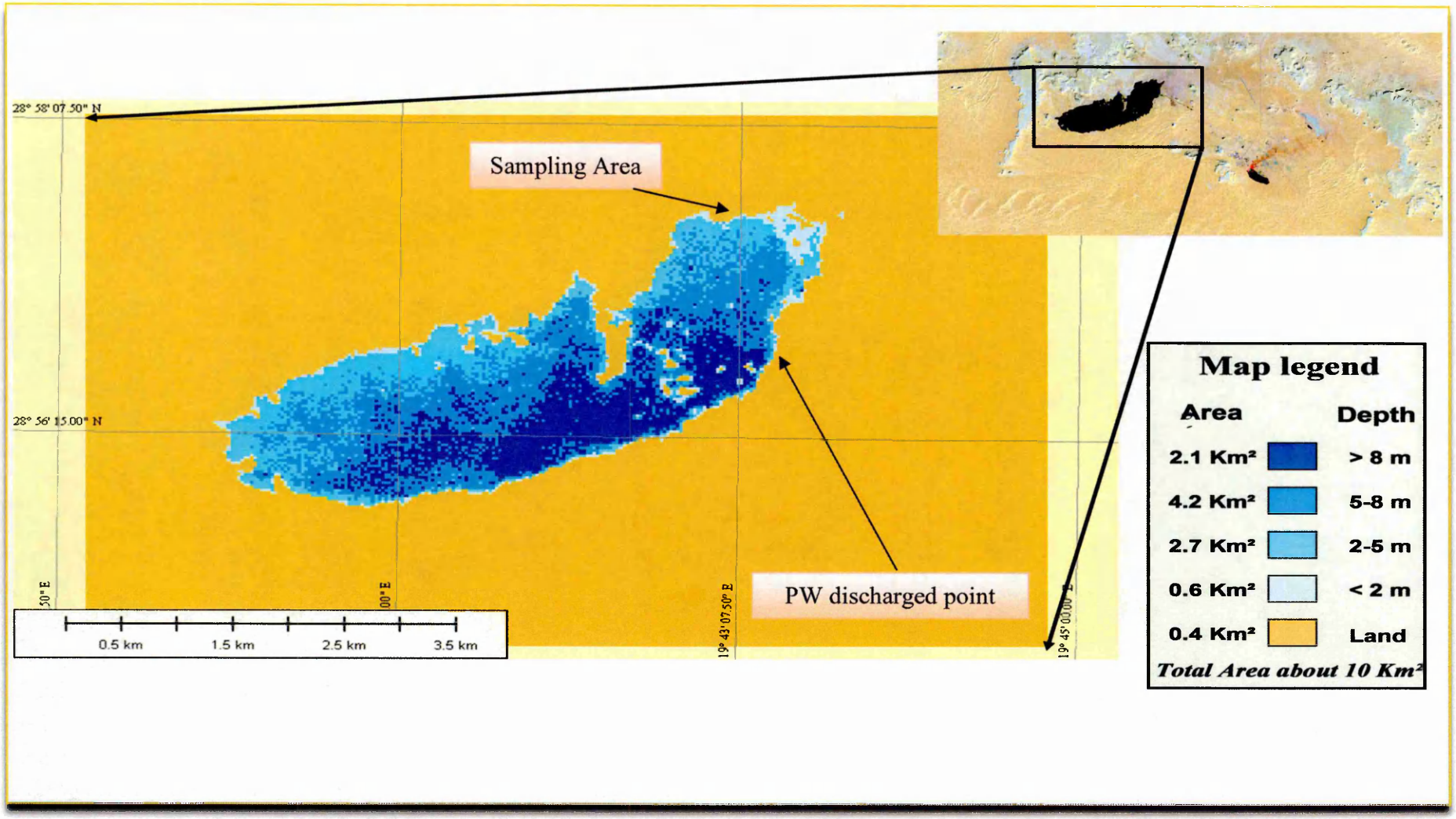


Figure 2-14 Size and depth of the evaporation pit at the Nasser oilfield (2008)

Table 2-1 Quantities of water produced in the Nasser Oilfield during the period from 1999 to 2008

Years	Produced water (mb/y)	Produced water (m^3/y)
1999	77293500	9216513
2000	80865390	9642427
2001	87481380	10431321
2002	100335500	11964052
2003	110208700	13141337
2004	108715300	12963263
2005	113393300	13521071
2006	114908100	13701696
2007	115522500	13774957
2008	116289000	13866355
Total	**1.025×10^9**	**1.22×10^8**

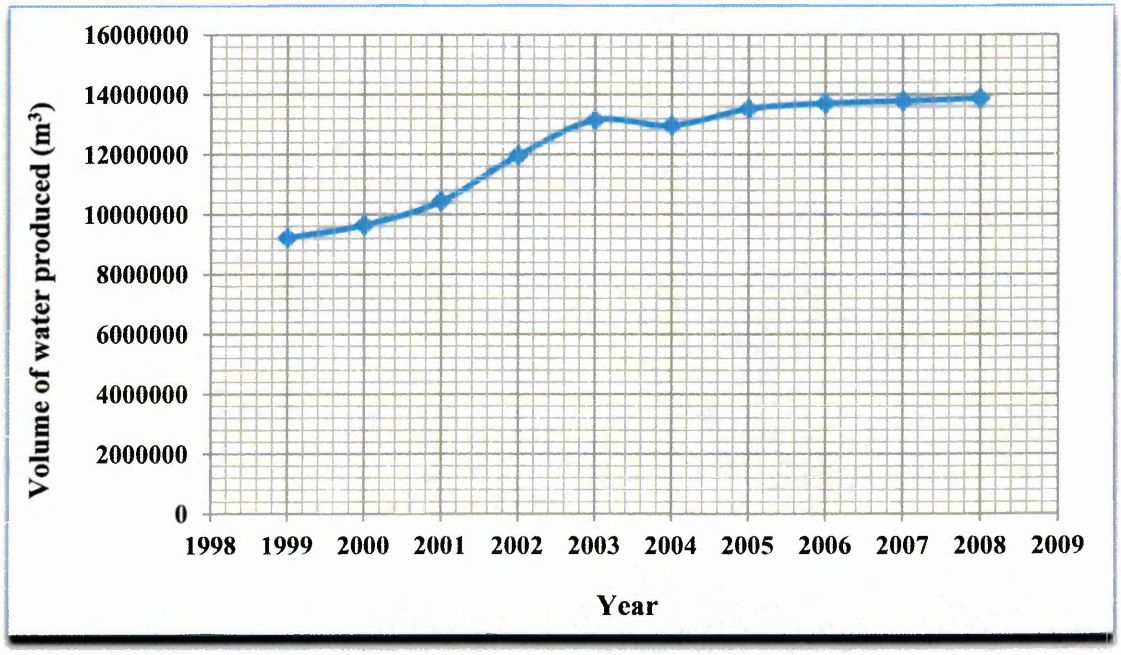


Figure 2-15 Produced water production profile for the Nasser oilfield during the period from 1999 to 2008

2.11.3 Sampling

The collection and preparation of samples are important aspects of any assessment program. The samples must be taken in a way that will avoid the introduction of bias systematic or non-systematic errors. The methods of collection and sample size should be chosen to ensure that the samples obtained are representative of the environment from which they are taken. Physical techniques can be applied to make the sample homogeneous such that further sub-sampling will ensure the material taken for analysis is wholly representative.

2.11.4 Sampling strategy

The main objectives for a sampling program at this site are to:-

- ❖ Collect PW samples for full analysis to characterize the main constituents that contributed to the environment contamination.
- ❖ Obtain soil samples for full analysis to characterize the contaminants.
- ❖ Obtain crude oil samples.
- ❖ Obtain samples of OFCs (corrosion, scale and demilsfier inhibitors).

2.11.5 Sample collection, storage and preservation

This information that is obtained from the site visit and site investigation can be used to characterize the site in order to determine the extent of the contamination. The chemical techniques used represent one of the main sources of information obtained to find out the nature of the contaminant in the soil and PW if there is any.

Samples were taken according to USEPA protocols to ensure the reliability of the analytical results. Procedures included the use of precleaned and sealed glassware.

2.11.6 Sample collection

2.11.6.1 Soil samples

Baseline soil samples for contaminant concentrations, soil chemistry and microbiology were collected from aged contaminated soils around the site. Soil samples were collected to evaluate the impacts on soil chemical properties.

One bank of the PW disposal pit was chosen as representative of the polluted area, Figure 2-16. It is periodically covered by PW and dried. The area selected was divided into 18 equal sampling units of area 10 m × 5 m each as shown in Figures 2-17 & 2-18. Using a hand shovel, random soil samples were scooped out to a depth of about 0- 20 cm within each unit. A total of 18 soil samples were collected. Two samples of uncontaminated soil were taken to be used as a reference and one aged contaminated soil sample was collected from an evaporation pit that has not been used for more than

20 years. Table 2-2 shows the location of the sample when it was taken and codes given to the soil samples.

Figure 2-16 Produced water disposal pit at the Nasser oilfield, Libya

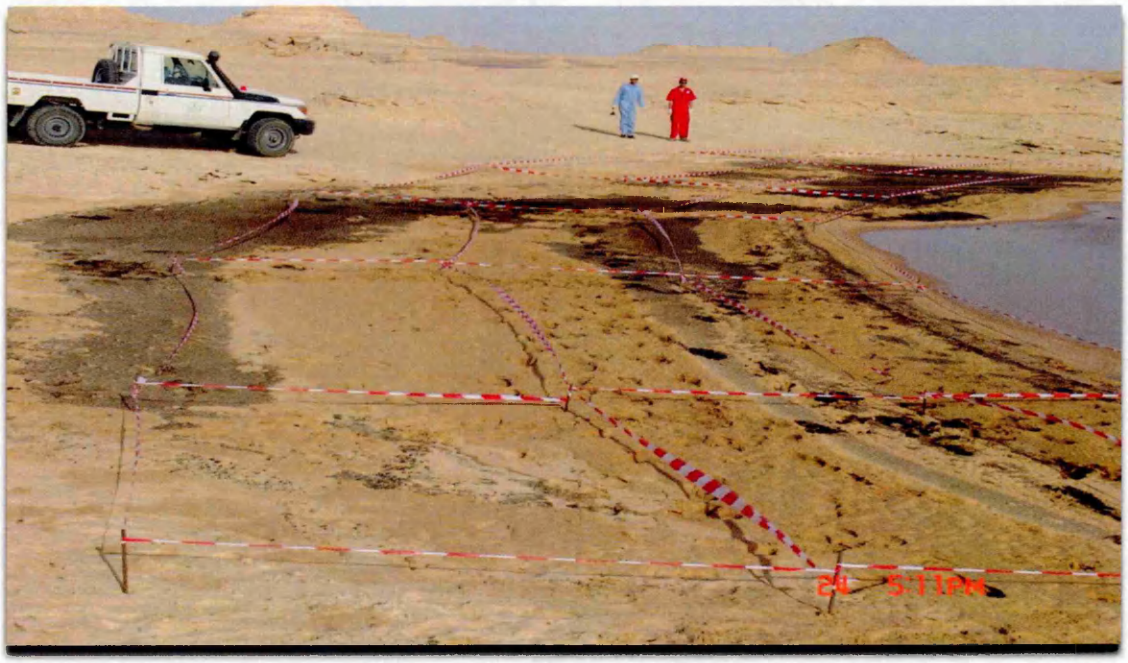


Figure 2-17 Delineated land at the bank of the produced-water disposal pit at the Nasser oilfield, Libya

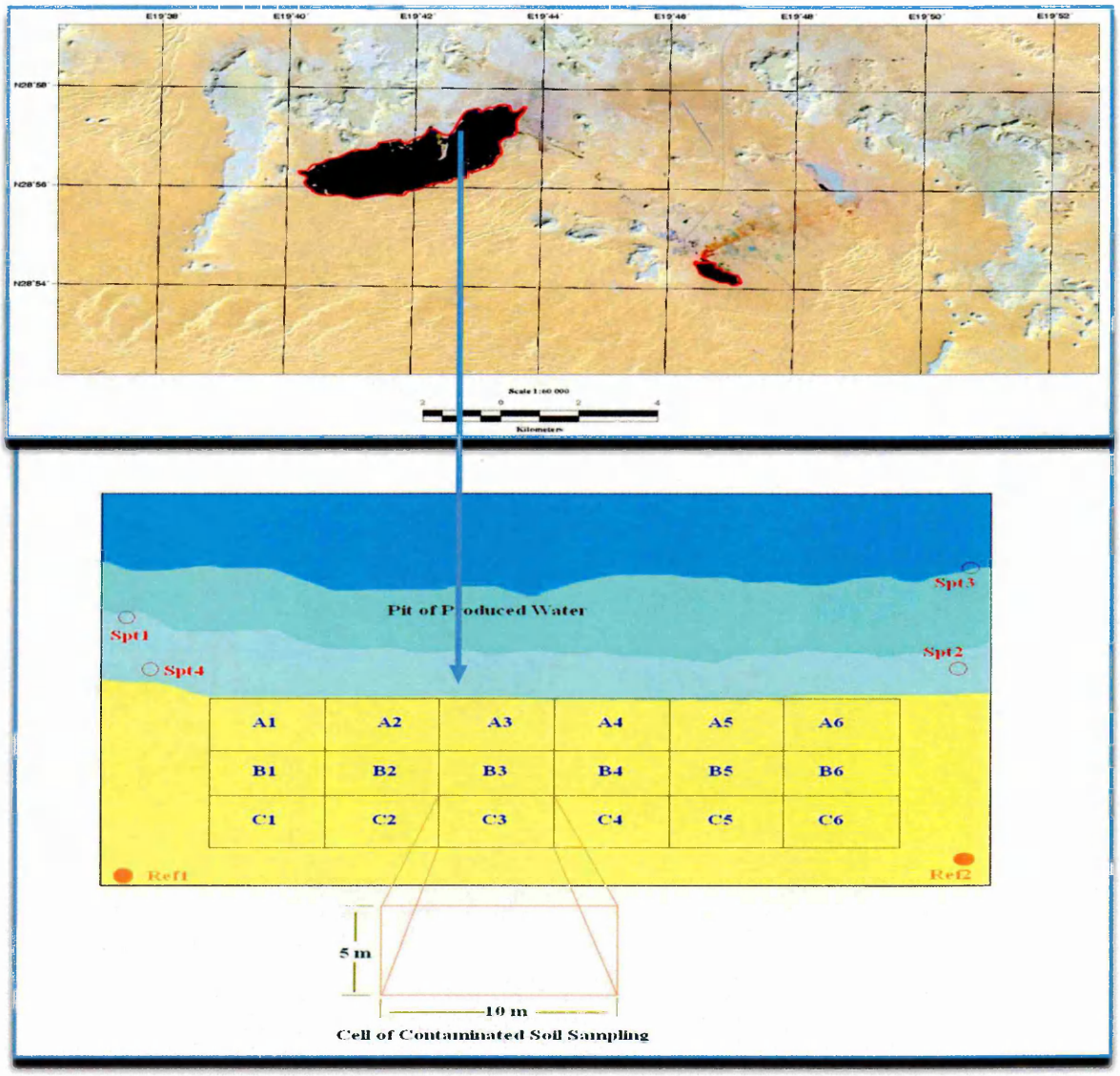


Figure 2-18 Schematic showing pit delineated by unit, sites within units and satellite image of the pit and the locations of produced water and soil sampling points around the pit

Table 2-2 Sampling points, locations and codes given to soil sample

Sample code	Sample description
A1 ~ A6	1st row of contaminated soil samples
B1 ~ B6	2nd row of contaminated soil samples
C1 ~ C6	3rd row of contaminated soil samples
Ref. 1 ~ 2	Uncontaminated soil samples (reference samples)

2.11.6.2 Produced water samples

Six samples of PW were collected namely TA, Pump, Spt1, Spt2, Spt3 and Spt4. Table 2-3 shows the locations of sampling point and codes given to the PW samples.

Table 2-3 Sampling points, locations and codes given to produced water sample

Sample Code	Sampling Points	Sample Type
TA	Separation Tank	Produced Water
Pump	Water pump station	Produced water (pumping point)
Spt1	Evaporation pit	Produced water collected from pit
Spt2	Evaporation pit	Produced water collected from pit
Spt3	Evaporation pit	Produced water collected from pit
Spt4	Evaporation pit	Produced water collected from pit

2.11.6.3 Crude oil

Two crude oil samples were taken, one from the storage tank, and the other from the disposal pit, in order to identify the carbon distribution. This is considered as one of the main pollutants in the soil.

2.11.6.4 Oilfield chemicals (OFCs)

The oil processes, production and separation involve addition of a different mixture of OFCs to the oil–water mixtures. As an example, scale inhibitors are added to prevent mineral scale deposition blocking pipe work, corrosion inhibitors are added to prevent the pipe work from attack by salt water and dissolved gases, biocides are added to prevent bacterial degradation of the oil and other products, and demulsifiers to help oil–water separation. Some or all of these oilfield chemicals may be discharged to the environment along with the PW. Seven Samples of OFCs used at the site namely B6206, C1180, C1053, C2772, D6261, S2510 and S6165 were taken in order to identify the chemical species present within them.

2.12 Field tests

After sample collection, immediate analyses were carried out in order to characterize the parameters (i.e., pH, T, EC and H_2S) that might change on storage. Preservation procedures were also carried out in order to control the main properties and inhibit the

interferences. All samples were collected using sampling techniques specified by standard methods and then the samples were transferred to the laboratories for investigation.

Primary analyses; temperature, pH, electrical conductivity and hydrogen sulfide content were carried out "on site" for PW samples in order to identify the range of some properties and to have an idea of impaction extent. The "on-site" field analyses are summarized in Tables 2-4.

Table 2-4 Physical properties and hydrogen sulphide of produced water

Sample Code	pH	EC (µmhos/cm)	Temperature (°C)	H_2S (mg/l)
TA	6.9	20500	53	259.25
Pump	7.1	23600	55	267.75
Spt1	6.6	195977	27	11.10
Spt2	6.6	189254	26	10.80
Spt3	6.4	187623	27	10.00
Spt4	6.7	193986	27	11. 00

2.13 Conclusions

PW is one of the most important sources of pollution associated with crude oil and natural gas production. The large volume (315,000 barrels per day) of oily PW generated from the Nasser oilfield and directly discharged to the soil surface into open environment cause an environmental problem to the ecosystem and could be a possible health risk for local people.

The purpose of this chapter was to obtain an overall picture of the contamination present on the site by visual observation, photographic documentation, sampling and then chemical analysis. In order to examine the potential impacts arising from exploration and production it is important to understand the sources and nature of contaminates. The most important conclusions which could be drawn from this chapter are summarized in the following points:-

- According to field investigations during the site visit, the site is highly polluted by oily PW disposal.
- The soil around the pits (about 20 meter from the pits edge) is definitely impacted, due to PW depositing. The impact has been recognized and ensured by visual observations.
- The satellite scenes were acquired within a period of 34 years (1972-2006) at the Nasser Oilfield during a different pumping rate. The results show that the expansion and depth of the pit can be estimated over very large areas via satellite data. Satellite records of pit water sizes give you a good indication of whether there is going to be a systematic or major problem in water supply. The study shows that satellite images aid in showing what cannot be measured or seen by other traditional techniques where the changes have been measured accurately during the period from 1972 till 2006. The satellite images are proved very effective in this type of monitoring where managed to come to the following results.
    - A classified map of the main pit under study shows the distribution of the depths with their sizes.
    - This classified map reveals that the shallowest areas located at the margins of the pit with the depth less than 2 meters and increasing gradually into the south central part of the pit colored as dark blue with depth more than 8 meters.
    - The map illustrates the different sizes with different depths where the largest area of the pit about 4.2 km^2 with a depth

from 5-8 meters while the shallowest area is about 0.6 km^2 with a depth less than 2 meters. The whole size of the pit covers an area of about 11 km^2

- Hydrogen sulfide (H_2S) is a corrosive and toxic gas. It is naturally generated and emitted by sulphate-reducing bacteria growing in subsurface formations and oilfield surface equipment. The evolution of hydrogen sulphide is currently inhibited by using powerful biocides like acrolein and formaldehyde; unfortunately these biocides are highly toxic and dangerous materials, so that they should be handled and treated carefully. Concentration of H_2S was detected and determined in produced water which considered being a serious challenge for human health as well as equipments used in production process.

Chapter Three
Physiochemical composition of produced water and its impact on soil

3.0 Introduction

As explained previously, the production of crude oil and natural gas from oilfields is usually associated with varying quantities of water called FW. This has been present along with crude oil in the reservoir for millions of years (Dunger et al., 1996, Obire et.al., 2003 and Okoro 2008). Once this water is brought to the surface with crude oil it is called PW. The volume of PW extracted is normally low in the early stage of oil production. The volume increase to as high as 90 % of the total volume (McCormack et. al., 2001 and Ebrahimi et al., 2012). The quantity of PW during the field lifetime of the oilfield can range from seven to ten times the amount of oil produced. Again, this represents the largest waste stream in most crude oil and natural gas activity (Dorea et al., 2007 and Horner et al., 2012). It can be disposed of by returning by injection into the reservoir formations, so environmentally hazardous wastes are cleaned and the pressure is raised in the reservoir, i.e oil is pushed out of the reservoir into the operating wells. Historically, some of the PW from oilfields is re-injected into producing zones to maintain reservoir pressure and to displace oil to production wells; this is called secondary recovery (i.e. enhanced oil recovery), injection into the deep well formations for safe disposal is also carried out and the remaining water was discharged into open environment (Sirivedhin et al., 2004 and Lu et al., 2006).

The physical and chemical properties of PW depend on the geographical location of oilfields, the geological formations from where it was produced, and the type of hydrocarbon product being produced. (Woodall et al., 2001, Veil et al., 2004 Yeung et al., 2011and Horner et al., 2012). PW properties and volumes vary significantly due to the injection of additional water into the formation to increase hydrocarbon production. PW is characterized as saline water with high concentrations of total dissolved solids (TDS) and other inorganic constituents. Inorganic constituents present in PW are mostly derived from the reservoir geology (Woodall et al., 2001, Yeung et al., 2011 and Wang et al., 2012). PW contains a wide range of dissolved and suspended materials include: cations [Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Fe^{2+}], anions [Cl^-, Br^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, NO_3^-, OH^- and PO_4^{3-}], fatty acids [formic, acetic, etc.], dissolved gases [CO_2, H_2S], hydrocarbon [oil-free, dispersed and dissolved] and OFCs (Lu et al., 2006).

As it was mentioned in the introductory chapter, PW has various potential environmental effects depending on where it is discharged. Oilfield brine (i.e. PW) impacted soil is the most common environmental problem associated with oil production, because it contains high salt content. The impacts of the disposal of PW are apparent in salt scars; this causes the death of trees and other vegetation and

contaminates soil and surface water. In addition, impacted soil leads to plumes of saline water that affect groundwater supplies and shallow aquifers (Abdol Hamid et al., 2008). Salts accumulating in soils can also result in three types of soils: saline, saline-sodic and sodic. Saline soils are the easiest to correct; sodic soils are more difficult (Qadir et al., 2004). When the salts build-up in soil problems arise for two main reasons: the soil becomes less permeable, and the salt damages or kills the plants. In sodic soils, high levels of exchangeable sodium cause the individual sand, silt and clay particles to be separated and not clumped together into larger particles. This spreading makes the soil tight and impervious, so that it lets little air or rain to permeate into the soil (Khosla et al., 1979 and Pitt et al., 2008).

This chapter describes the full characterization of the physical and chemical properties of PW and PW impacted soil from the Nasser Oilfield, where the bank of the disposal pit has been chosen as a case study area (Figures 2-17 and 2-18). Analysis of both the PW and the impacted soil samples have been carried out. The main constituents that cause pollution of the soil have been identified. The analyses results have been used to recommend a plan of soil remediation. The principle objective was to determine how effective state of the art technology is in producing acceptable quality effluents in PW from oil production operations. Additionally, an attempt was made to generate comprehensive information on the chemical composition of PW and compositional changes in the process stream. This analysis covers a range of anions and cations present in the soil and produced water. Soil soluble salts are often compared to a combination of major elements including the Na^+, K^+, Ca^{2+} , Mg^{2+} , Cl^-, HCO_3^-, CO_3^{2-}, SO_4^{2-}, and trace elements. In addition a study of widespread contamination of soil caused by PW disposal on soil, and characterization of contamination, fate, transport of contaminants in soil round the oilfield of the case study has been completed.

3.1 Materials and methods

The area selected was divided into 18 equal sampling units as shown in Figures 2-17 and 2-18. A total of 18 soil samples and two samples of uncontaminated soil far away from the polluted area to be used as a reference were collected. In addition six produced water samples and one groundwater samples were collected. One sample from settling tanks (TA), one sample from pumping station (Pump) and four (Spt1 to Spt4) from disposal pit were also collected as well, all the collected samples were preserved in accordance to international standards and transported to the laboratory and stored under refrigeration until analysis.

Many parameters have been determined for both soil and produced water samples in order to characterize the polluted site and to develop guidelines for soil restoration and remediation.

I. Soil samples tests: 100 g of soil samples were air dried and passed through a 10-mesh sieve and an equal amount of deionised water (100 ml) were added to the flask, then mechanical shaken for 30 minutes. The contents were allowed to stand, then the contents were filtered and the extract or supernatant were analysed for pH, electrical conductivity (EC), anions and cations and total dissolved solid (TDS). The cations are used to calculate the Sodium Adsorption Ratio (SAR).

II. Produced water samples tests: direct pH and EC were determined as well as cations & anions and TDS were determined according to ASTM procedures.

Test methods used for physio-chemical analysis of soil and PW cover the determinations of pH, EC, Na^+, K^+, Ca^{2+} , Mg^{2+} , Cl^-, HCO_3^-, CO_3^{2-}, SO_4^{2-}, hardness, TDS and trace elements in soil and PW. The PW was characterized as brackish groundwater with elevated concentration of dissolved salts. The majorities of the analyses used out throughout the study have been carried out according standard method (ASTM or published methods) as described below and given in Table 3-1.

3.2 Reagents

All chemicals and reagents used in this investigation were of analytical grade and conform to the specification of the committee on analytical reagents of the American Chemical Society. All water used was Milli-Q grade.

3.3 Physical properties

3.3.1 pH measurement

pH is the measure of the acidity or basicity of a water. pH is measured on a scale that runs from 0 to 14. Clean water is said to be neutral with a pH close to 7.0 at 25°C, this means that there is balance between acid and alkalinity. Water with pH less than 7 means acid is present and a pH above 7 is basic or alkaline. The pH measurements are important in food science, biology, chemistry, environmental science and many other applications. The Environmental Protection Agency (EPA) recommends that water systems maintain pH levels of between 6.5 and 8.5. Acidic water can leach metals from pipes. It also can damage metal pipes and cause aesthetic problems. Water with high pH can cause aesthetic problems such as an alkali taste to the water; scale build up in plumbing; and lowered efficiency of electric water heaters (Abrol et al., 1988). The objective of these experiments is to identify and define the possible impact of PW on the acidity and alkalinity of topsoil. A standard method (ASTM D1293-99, Reapproved

2005) commonly used for measuring pH of solution was employed. The pH was performed immediately after the receipt of sample in the laboratory; this is due to the fact that it is subjected to interferences such as sedimentation, oxidation and hydrolysis.

Table 3-1 A summary of the analytical procedure used

NO.	PARAMETER	UNITS	METHOD
1	pH value @ 25°C	-	ASTM D - 1293
2	(E.C) Electrical conductivity	µS/cm	ASTM D – 1125
3	Resistivity	m/ohm	ASTM D – 1125
4	Sulphates (SO_4^-)	mg/l	ASTM D – 516
5	Methyl orange alk as $CaCO_3$	mg/l	ASTM D – 1067
6	Sodium Na^+	mg/l	ASTM D – 2791
7	Potassium K^+	mg/l	ASTM D – 2791
8	Total hardness as $CaCO_3$	mg/l	ASTM D – 1126
9	Calcium hardness as $CaCO_3$	mg/l	ASTM D – 1126
10	Magnesium hardness as $CaCO_3$	mg/l	ASTM D – 1126
11	Calcium	mg/l	ASTM D – 511
12	Magnesium	mg/l	ASTM D – 511
13	CO_3^{2-}	mg/l	ASTM D – 1067
14	HCO_3^-	mg/l	ASTM D – 1067
15	Chloride Cl^-	mg/l	ASTM D 4458-09
16	Salinity calculated as NaCl	mg/l	calculation
17	Dissolved solids evap. @ 180°C	mg/l	ASTM D – 5907

3.3.1.1 Instrument and method used

The pH values were measured using a glass electrode connected to a Knick digital pH meter 646, (Berlin). The pH meter was standardized with three singlet pH buffer solutions (pH 4, pH 7 and pH 10 respectively). The temperature of the solution was entered digitally before the pH of the sample was measured according to a standard method (ASTM D1293- 99, Reapproved, 2005).

3.3.1.2 Procedure

The pH of the sample with the same temperature as the buffer solution was measured by putting the sample and the electrode into the glass beaker whilst stirring the pH value taken as there was no fluctuation observed. Then the electrode was rinsed with de-ionized water and dried after that immersed into buffer solution.

3.3.2 Electrical conductivity (EC) measurement

EC is a measure of the ability of water to conduct an electric current and depends on the concentration of ions (higher concentration, higher EC), temperature of the solution

(high temperature, higher EC) and specific nature of the ions (higher specific and higher valence, higher EC) present. The E.C is a function of temperature (Hayashi 2004), where the ionic velocities increase with the increase of temperature, due to this fact the E.C is always reported at the reference temperature of 25°C. The electrical conductivity is expressed in µS/cm (micro Siemens per cm) or dS/m (deci Siemens per m) Where: 1000 µS/cm = 1 dS/m. On the other hand EC estimates the amount of TDS, or the total amount of dissolved ions in the water. TDS, in milligrams per liter, is equivalent to approximately 640 times EC, in dS/m (www.salinitymanagement.org/.../ls/ls_3d.html and Hayashi 2004). EC can be converted to TDS using the following calculation:

$$\text{TDS (ppm)} = 0.64 \times \text{EC (µS/cm)}$$

The EC of water is actually a measure of salinity. So the EC of water is a good indicator of total salinity (Chhabra, 1996). Excessively high salinity can affect plants in several ways; a particular ion (such as sodium) may be toxic or higher osmotic pressure around the roots might prevent an efficient water absorption by the plant. This procedure applies to aqueous phase samples including PW, pit liquids and saturated paste extracts (Clesceri et al., 2005).

3.3.2.1 Instrument and method used

The EC was measured by a conductivity meter type LF191. This was calibrated by using reference standard solution (Model: EC215, HANNA, Italy) at 25°C, and was measured according to standard method (ASTM D1125–95 Reapproved 2009).

3.3.2.2 Procedure

The conductivity cell was rinsed with deionized water and filled with calibration standard, then the conductivity was read and recorded, after that the cell was rinsed with deionized water and filled with the sample then the conductivity was read and recorded.

3.4 Chemical properties

3.4.1 TDS measurement

Total Dissolved Solids (TDS) refers to any minerals, salts, metals, cations or anions dissolved in water, and is expressed in units of mg per unit volume of water (mg/l) (Clesceri et al., 2005). TDS comprise inorganic salts (calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates) and some small amounts of organic matter that are dissolved in water, or TDS are any dissolved substances which remain after evaporation of the water at a fixed temperature which is usually 180°C. Therefore TDS is directly related to the purity of water and the quality of water purification systems and affects everything that consumes, lives in, or uses water. TDS can be

measured by two principal methods gravimetric and conductivity methods (Clesceri et al., 2005). Gravimetric methods involve evaporating the liquid to dryness to leave a residue that can be weighed; these methods are generally the most accurate. EC of water is directly related to the concentration of dissolved ionized solids in the water. The relationship between TDS and conductivity is a function of the type and nature of the dissolved cations and anions in the water and possibly the nature of any suspended materials. TDS was measured according to (ASTM D5907-03). This procedure applies to aqueous phase samples including PW, pit liquids and saturated paste extracts. TDS is minerals matter passing a standard glass filter, and the remaining after drying at 180°C and weighted it to constant weight.

3.4.1.1 Procedure

A 0.45-µm filter was inserted into the assembled filtration equipment, then the vacuum was applied .The disc was washed three times with 90 ml of deionized water and this wash water was discarded. 100 ml of the sample was filtered and washed with 20 to 60 ml of deionized water, complete drying between washings was allowed. The filtrate aliquot was transferred to the (125 ml) weighed evaporation dish previously cleaned by ignition to 550°C for 1 hr and the filtrates were evaporated at 180°C over a water bath until dryness. The evaporation dish was dried for 1h at 180°C in the oven, and then allowed to cool down in a desiccator for 30 minutes prior to weighing as follows

$$\text{Total dissolved solid [TDS in mg/l]} = A\text{-}B / V \times 1000$$

Where

A= weight of dish plus residue (mg).

B= weight of dish (mg).

V= Sample volume (ml).

3.4.2 Total hardness, calcium and magnesium hardness as $CaCO_3$

Water which contains high levels of minerals is called hard water. A hard water mineral consist of calcium Ca^{2+} and magnesium Mg^{2+} and sometimes contains other dissolved compounds such as bicarbonates and sulphates. There are two types of water hardness: General hardness (GH) and Carbonate hardness (CH). A third term commonly used is total hardness which is a combination of GH and CH. The hardness of water is a measurement of the total concentration of the calcium and magnesium ions and is expressed as calcium carbonate $CaCO_3$ (Clesceri et al., 2005). Total hardness, calcium hardness and magnesium hardness as $CaCO_3$ (mg/l) were measured by titration with EDTA according to standard methods (ASTM D1126 – 02, Reapproved 2007).

3.4.2.1 Total hardness as $CaCO_3$ (mg/l)

A 100 ml water sample was measured into flash with continued stirring. The pH of the sample was adjusted between 7 to 10 by adding NH_4OH or HCl solution. Then 1 ml volume of buffer solution and 0.2 g of hardness indicator powder pillows were added to the water sample and stirred, a red colour resulted. The sample was titrated with 0.01M Na_2H_2EDTA standard solution until the colour changed from red to blue and then after 5 min of the end point the volume of standard solution used was recorded and the concentration was calculated as follows:

$$\text{Hardness (mg/l) as } CaCO_3 = V1 / V2 \times 1000$$

Where

V1 = Volume of Na_2H_2 EDTA used in ml.

V2 = Volume of water sample in ml.

3.4.2.2 Calcium hardness as $CaCO_3$ (mg/l)

50 ml of the sample was transferred into 200 ml beaker then 2 ml of NaOH solution and 0.2 g of calcium indicator were added. Then the mixture was titrated with Na_2H_2 EDTA solution with continued stirring till the colour was changed from red to royal blue. After the titration was complete the volume of standard was recorded and the concentration was calculated as follows:

$$\text{Calcium hardness (mg/l) as } CaCO_3 = V1 / V2 \times 1000$$

Where

V1 = Volume of Na_2H_2 EDTA used in ml.

V2 = Volume of water sample in ml.

3.4.2.3 Magnesium hardness as $CaCO_3$ (mg/l)

Magnesium hardness = (Total hardness) − (Calcium hardness) as $CaCO_3$

3.4.3 Calcium and magnesium (Ca^{2+}, Mg^{2+}) determination

Calcium and magnesium are present in all water. The determination of alkaline earth metals, particularly calcium and magnesium is of importance in environmental, biological and industrial applications. The Ca^{2+} and Mg^{2+} were measured in water by titration according to standard method (ASTM D511-03 Reapproved 2009).

3.4.3.1 Procedure

100 ml of mixed acidified sample were transferred into a 150 ml beaker and 5 ml of HCl was added. The sample was heated on a steam bath until the volume reduced to 20 ml, then the sample was cooled and passed through ashless filter paper into a 100 ml volumetric flask. A 50 ml portion from the flask was taken and the pH was adjusted to

pH 7 to pH 10 with ammonium hydroxide (NH_4OH). 1 ml of $NH_2OH{\cdot}HCl$ solution was added then 1 ml of buffer solution (pH 10.0±0.1) and 2 ml of NaCN solution were added. Then one or two crystals of $K_4Fe(CN)_6{\cdot}3H_2O$ and 4 to 5 drops of Chrome Black T indicator were added. The sample was titrated with EDTA solution till the colour changed to clear blue and after 5 min the volume of EDTA solution required to titrate calcium plus magnesium was recorded. A reagent blank was titrated following the similar steps that have been followed in respect of calcium plus magnesium.

3.4.3.2 Calcium Ca^{2+} determination

The burette refilled with EDTA standard solution and 25 ml of filtered sample was transferred into 150 ml beaker then 1 ml of $NH_2OH{\cdot}HCl$ solution and 1 ml of NaOH were added and the pH was adjusted to pH 12 to 13. After adding 1ml of NaCN and 0.2 grams of calcium indicator (fluorescein methylene iminodiacetic acid) solution, the sample was titrated with standard EDTA, and the titration continued till the colour changed from deep green to purple. After 5 min of the titration the end point was recorded (volume of EDTA solution required to titrate calcium). A reagent blank correction was determined following the similar steps that were used to titrate calcium. The concentration of calcium and magnesium were calculated as follows:

$$\text{Calcium in (mg/l)} = A \times B / D \times 40100$$

$$\text{Magnesium in (mg/l)} = [(C \times B / E) - (A \times B / D)] \times 24300$$

Where:

A = EDTA standard solution required to titrate calcium in the sample) - (volume of EDTA consumed in titrating reagent blank correction)

B = Molarity of EDTA standard solution.

C = EDTA standard solution required to titrate Ca^{2+} and Mg^{2+} in the blank correction.

D = Sample volume (ml) to measure Calcium Ca^{2+}

E = Sample taken to measure Calcium $Ca^{2+}$and Magnesium Mg^{2+}

3.4.4 Sodium and potassium (Na^+ and K^+) determination

Na^+ and K^+ are alkali metals and are chemically very similar. Potassium and sodium ions are necessary for the function of all living cells, and are thus present in all water, plant and animal tissues (Clesceri et al., 2005). Na^+ and K^+ in samples were measured by using flame photometry (CORNING 400, Corning medical & Scientific, England, UK), according to standard method (ASTM D2791–93 Reapproved 2001).

Na^+ and K^+ calibration standards (0, 2, 5, 10, 15, 100 ppm) were prepared. The emission was measured using flame photometry starting from highest concentration and working

towards the most diluted one for Na^+ and K^+ standard; then the samples were measured the same way as the standards and the of Na^+ and K^+ concentrations were read from calibration curves of the standard solutions.

3.4.5 Chloride (Cl^-) determination

Chloride is an ionized form of chlorine, because most chloride salts are highly soluble, chloride is one of the most common ions found in waters. The chloride ion does not react with, or adsorb to most components of rocks and soil, and is easily transported through the soil column (Clesceri et al., 2005). Therefore chloride is an effective tracer for pollution from disposal of waste water or for salt water intrusion. The measurement of chloride is possible in highly mineralized waters such as oilfield brines, seawater, and brackish water. The method is based upon the titration of chloride with silver nitrate ($AgNO_3$), according to standard method (ASTM D4458 – 09).

3.4.5.1 Procedure

50 ml of sample was filtered and transferred to a 150 ml conical flask and diluted with 100 ml water. The pH was adjusted to between 6.5 to 8.0 by adding 1 g of sodium bicarbonate and stirred. 1 ml of 5 % chromate indicator was used as an indicator and titrates of with silver nitrate ($AgNO_3$) solution. The titration was continued till the colour permanent orange colour preceding the brick red precipitate was observed and the volume of $AgNO_3$ was recorded and the concentration of chloride was calculated as follows:

$$\text{Chloride in (mg/l)} = V\text{-}B \times T \times 1000 / C$$

Where

V= volume of $AgNO_3$ standard used

C= sample volume used

T = titre, mg Cl^-/ml of $AgNO_3$ and

B = indicator blank

3.4.6 Sulphate (SO_4^{2-}) determination

SO_4^{2-} is a naturally occurring substance that contains sulphur and oxygen. It is present in various mineral salts that are found in soil. SO_4^{2-} forms salts with a variety of elements including barium, calcium, magnesium, potassium and sodium. Sodium, potassium and magnesium sulphate are all soluble in water, whereas calcium and barium sulphates and the heavy metal sulphates are not. Dissolved sulphate may be reduced to sulphide, volatilized to the air as H_2S, precipitated as an insoluble salt or incorporated in living organisms. SO_4^{2-} may be leached from the soil and is commonly found in all natural water (Clesceri et al., 2005). Sulphates are discharged into the

aquatic environment in wastes from industries containing high SO_4^{2-} concentration. SO_4^{2-} is generally considered to be non-toxic. Water containing high amounts of magnesium or sodium sulphate may result in intestinal discomfort, diarrhea and consequently dehydration. It is not advisable to use water that contains high concentrations of sulphate. SO_4^{2-} in aqueous solutions may be determined by a gravimetric method in which sulphate is precipitated as barium sulphate; according to standard method (ASTM D 516-07).

3.4.6.1 Procedure

100 ml of the sample was filtered throughout a 0.45-μm filter paper, 5 ml of conditioning reagent and the solution were mixed and stirred, and then 0.3 g of $BaCl_2$ was added while stirring vigorously. The temperature was kept below boiling until the liquid became clear and the precipitate was settled completely for 2 h .The suspension of $BaSO_4$ was filtered on fine ashless filter paper and the precipitate was washed with hot water until the washing was free of chlorides as indicated by testing the last portion of the washing with $AgNO_3$ solution. The filter paper and the contents were placed in a weighed platinum crucible and charred without flaming. The residue was ignited at about 800°C for 1 h, then a few drops of H_2SO_4 and HF were added and the solution was evaporated under a hood to expel silica as silicon tetrafluoride (SiF_4). The residue was ignited at about 800°C, cooled in a desiccator and the $BaSO_4$ weighted. The SO_4 was calculated according the following equation:

$$\text{Sulphate in mg/l} = W \times F \times 1000 / S$$

Where, W= Weight of Barium Sulphate in mg

F= Analytical factor of SO_4 calculated from SO_4 / $BaSO_4$ = 0.4115

S = Sample volume used

3.4.7 Carbonate (CO_3^{2-}) /Bicarbonate (HCO_3^-) determination

The carbonate ion is the simplest oxocarbon anion. It is consists of one carbon atom surrounded by three identical oxygen atoms, it is trigonal planar. The bicarbonate ion is an anion with HCO_3^- ; it consists of one central carbon atom surrounded by three oxygen atom in a trigonal planar arrangement with a hydrogen atom attached to one of the oxygens. Natural waters contain a variety of weak acids and bases which include the major elements present in living organisms. By far the most important of these is carbon in the form of CO_2, HCO_3^- and CO_3^{2-}. Bicarbonates usually are present in oilfield water and often define the alkalinity. Alkalinity is a measure of the ability of a solution to neutralize acids to the equivalent point of CO_3^{2-} or HCO_3^- (Clesceri et al., 2005). The

alkalinity, CO_3^{2-} and HCO_3^- were measured by titrating a standard acid solution according to standard method (ASTM D1067 – 06).

3.4.7.1 Procedure

100 ml of the water sample was transferred into a 300 ml glass beaker at room temperature and gently titrated to the end point with 0.02M H_2SO_4 in the presence of 3 drops of Phenolphthalein as an indicator solution and stirring was continued until the titration was complete. Then the volume of standard used was recorded. If the colour did not change the sample did not contain carbonate. Bicarbonate was determined by the method as carbonate. A few drop of methyl orange indicator solution was added and the sample was titrated with standard solution until the colour changed from pink-yellow to pink-orange. Then the volume of standard used was recorded at the end point and the concentration was calculated as follows:

$$HCO_3^- \text{ (in meq/l)} = V1 \times N \times 1000 / V2$$

Where

V1= Volume of standard used in ml

V2= Volume of sample used in ml

N= Normality of H_2SO_4

$$\text{mg/l of } HCO_3^- = HCO_3^- \text{ (in meq/l)} \times 61 \text{ (MW of } HCO_3^-)$$

3.4.8 Salinity measurement

Salinity is the increased accumulation of excessive salts in land and water at sufficient levels to impact on human and ecosystems (Clesceri et al., 2005). It is a general term used to describe the level of different salts such as sodium chloride, magnesium and calcium sulphate and bicarbonate. Increasing salinity is one of the most significant environmental problems. Salinity becomes a concern when an "excessive" amount or concentration of soluble salts occurs in the soil, either naturally or as a result of contamination. It is important to identify saline areas so they can be appropriately managed. There are a range of methods for measuring salinity. Two common ways are by using an EC meter or by measuring how much salt is in a solution of soil or water according to the following equations.

$$\text{Salinity in ppm} = \text{Chloride} \times 1.8060 \text{ (Millero, et al., 2008)}$$

$$\text{Salinity calculated as NaCl in ppm} = (Cl\times1) + (Na\times1) + (K\times1) + (Ca\times0.95) + (Mg\times2) + (SO_4\times0.5) + (HCO_3\times0.27) + (CO_3\times1.6)$$

3.4.9 Sodium Adsorption Ratio (SAR) measurement

Sodium Adsorption Ratio (SAR) is based on the sodium, calcium and magnesium concentration in solution. SAR is a measure of the suitability of water for use in agricultural irrigation as determined by the concentrations of solids dissolved in the water. It is also a measure of the sodicity of soil, as determined from analysis of water extracted from the soil (Chhabra 1996 and Rashidi et al., 2008), which indicates soil impact by sodium element and the changed in structure caused by this impaction where the porosity and permeability are decreased. The importance of measuring SAR is demonstrated by the range of the effects on surface soil by salt especially sodium salt. The SAR is defined as the milliequivalent weight of sodium divided by the square root of the sum of the milliequivalent weights of calcium and magnesium, divided by 2, (Chhabra 1996 and Rashidi et al., 2008), or in equation format:

$$SAR = Na^{+} / [(Ca^{+2} + Mg^{+2}) / 2]^{1/2}$$

Na, Ca, and Mg expressed as meq/l

Conversion to meq/l

Na, meq/l = Na in mg/l / 23

Ca, meq/l = Ca in mg/l / 20

Mg, meq/l = Mg in mg/l / 12

3.4.10 Trace elements analysis in soil and produced water

PW which is disposed from crude oil operations usually contains certain non-hydrocarbon components and trace elements (Moatar et al., 2010). PW is usually disposed to the environment into disposal ponds near the oilfield containing some of these trace elements. Concern over the adverse human and ecological health effects of the increasing accumulation of heavy metal contaminants in the environment are growing. Heavy metal pollution accumulated in soil influences the ecosystem nearby. The toxic metals can be taken up directly by humans and animals through the inhalation of dusty soil or they may enter the food chain as a result of their uptake by plants, animals, or leach down to groundwater and contaminate drinking water resources, and may cause, hazards to the health of humans and animals. PW and soil samples from disposal ponds at the Nasser oilfield were collected from the site and transported to the laboratory. An acid digestion procedure was used to prepare the soil sample for analysis. PW samples were filtered through a 0.45 μm filter and acidified to a pH of 2.0

with HNO_3. The concentrations of 15 metals (As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Se, Sr and Zn) in the soil and PW samples were analyzed using inductively coupled plasma optical emission spectrometer (ICP-OES).

3.4.10.1 Instrumentation

ICP-OES is a major technique for trace element analysis. The sample to be analyzed is first treated and mixed with water and passed it to the plasma, then the molecules in the sample are converted into individual atoms and ions by a high temperature radio frequency induced argon plasma (Boss et al., 1997). The sample is introduced into the plasma by pumping it using a peristaltic pump to a nebulizer and converted to a fine spray that is mixed with argon in a spray chamber. Then the sample is carried into the plasma and excited by the high temperature. Atoms became ionized 99 % (Boss et al., 1997). In OES, the intensity of the light emitted at specific wavelengths is measured and used to determine the concentrations of the elements of interest. The most important advantages of OES results from the excitation properties of the high temperature sources used in OES. These thermal excitation sources can populate a large number of different energy levels for numerous different elements at the same time. The analysis of metals in brine solutions present many difficult problems, the first concerns are problems with ionization from alkali and alkaline earth metals and the second when analyzing high salt concentration in the sample clogging of nebulazer and injector tubes can occurs (Boss et al., 1997). The brine samples often required longer rinse times at high frequency and dilution of samples are required for major constituent determination. The instrument used was an ICP-OES, Model VISTA - PRO, CCD Simultaneous ICP-OES, Varian). The instrument includes a CCD array detector with the ability to capture the entire wavelength spectrum in one reading without scanning. Vista-MPX software captures the entire spectral image in one reading, interferences are easily avoided by choosing any wavelength from 175-785 nm. Simultaneous background correction and internal standardization resulting in more accurate and precise results with excellent long term stability. The unique MPX CCD array detector is cooled to -30°C for the ultimate in low noise performance and best possible detection limits. The instrument was calibrated for the measurement of emission for standard solutions and a blank with multiple calibration curves element, standard solution to cover the wide range of metal concentration.

3.4.10.2 Reagents

All reagents were of analytical reagent grade. Water was doubly distilled and further purified using a Milli-Q water purification system. All calibration standard solutions

were prepared by diluting multi-element stock standards with 1 % v/v nitric acid (HNO_3), and prepared immediately before the measurement.

3.4.10.3 Determinations of trace elements in produced water sample

All glassware prior to use were soaked in 10 % v/v nitric acid (HNO_3) for several days then rinsed several times with deionized water. 100 ml of a well mixed water samples was preserved by acidification with 0.5 ml of conc. HNO_3 per liter of sample. The sample was transferred to a 150 ml beaker, and 5 ml of HCl was added, then the beaker was placed on the hot plate in a fume hood until the volume was reduced to about 20 ml by gentle heating for 2 hr, and then the beaker was removed from the hot plate and cooled and the sample was then transferred to a 100-ml volumetric flask, and the volume was adjusted to the mark, with deionised water, then the samples were analyzed using ICP-OES, Model VISTA - PRO, CCD Simultaneous ICP-OES, Varian, according to standard method (ASTM D1971-02 Reapproved 2007, ASTM C1111-04 and EPA Method 6010-C). Analyses of samples and standards were performed as soon as the preparation was complete.

3.4.10 .4 Determinations of trace elements in soil samples

The soil samples were dried for several days, sieved to less than 2 mm, and ground to a fine powder 0.5 g of soil was transferred to TEF-Fluorocarbon beaker and the blank was prepered at the same time as the sample in another beaker. The two beakers were then transferred to the hot plate at 200°C for 30min after the addition of 6 ml of HNO_3 to each beaker, then the samples were removed from the hot plate and 6 ml of hydrofluoric acid (HF) and 2ml of HNO_3 were added, then the beakers were return to the hot plate and heating was continued till the evaluation of white perchloric fumes and the solution became dry. Then the beakers were removed and after 5 min., 2 ml of perchloric acid ($HClO_4$) were added, heating continued until the solution became dry. Then the samples were removed and the temperatures of the hot plates were reduced to 100°C, 2 ml of HCl (1:1) and 10 ml of water were added and heating continued until the residue was dissolved. Then the beaker was removed and the solution was transferred to a 50 ml volumetric flask and the volume was adjusted with deionised water to the mark then the samples were analyzed by using an inductively coupled plasma optical emission spectrometer (ICP-OES). Analyses of samples and standards were performed as soon as the preparation was complete, according to standard method (ASTM D 4698-92 Reapproved 2001).

3.5 Results and discussion

Accidental releases of produced water (PW) and petroleum and the improper disposal of PW are national and oil and gas producer issues. The investigation described here covers the major physio-chemical parameters of PW and soil. It includes all the experiments intended and planned to assess the risk that the environment and the natural resources have been subjected to it. All experiments aim to determine the physical and chemical composition of PW and soil samples that have been collected from the Nasser oilfield, Libya.

The surrounding environment at oilfields usually suffers from the impact of pollution arising due to oil exploration, extraction and processing activities. A chemical analysis has been conducted on soil and PW samples, collected from a Libyan oilfield area, in order to detect and assay the pollutants. The pollutants found could be categorized into the following groups: cations, anions, trace elements, hydrocarbons, and OFCs.

The above groups of constituents are considered the important target for environmental studies due to their threat to the environment.

PW analyses are given in the ionic form, which is usually considered the best; and the interpretation is based on the ionic statement. Thus the common metallic bases sodium (Na^{+}), and (K^{+}), and the alkali earths calcium (Ca^{2+}) and magnesium (Mg^{2+}), are grouped as alkalies and are positive radicals. The acids or negative radicals consist of two groups the strong acids, sulphates (SO_4^{2-}), and chlorides (Cl^{-}), and the weak acids, carbonates (CO_3^{2-}) and bicarbonates (HCO_3^{-}).

3.5.1 Produced water samples

The full physico-chemical properties of the PW and groundwater samples from the Nasser oilfield, Libya including pH, conductivity, resistivity, total dissolved solids, salinity, alkalinity and hardness are presented in appendix A.

The results from the analysis of PW from the oilfield indicates that the pH values vary and range between 6.7 to 7.5, with an of average 7.2, as shown in Table 3-2. This is considered within the normal range, but it doesn't mean that the PW is unpolluted; it means that the dissolved constituents (both basic and acidic) are in an equilibrium state. The test is chosen to characterize the alkalinity and acidity of the study site. The pH of the water is not used for water identification or correlation purposes, but indicates possible scale forming or corrosion tendencies of water and also may indicate the presence of drilling mud filtrate or well treatment chemicals.

Table 3-2 Typical composition of produced water discharged and groundwater samples from the Nasser oilfield, Libya

Parameters	TA	PUMP	Spt1	Spt2	Spt3	Spt3	WW	Sea water*
pH	6.90	6.67	7.30	7.41	7.46	7.44	7.42	7.5- 8.5
EC (μScm^{-1})	37057	38066	196977	189292	187867	194313	6675	53880
Ca^{+2} (mg/l)	1348	1760	6720	6640	8160	8480	296	400
Mg^{+2} (mg/l)	476	389	1652	1604	1993	1993	190	1272
Na^{+} (mg/l)	8200	6650	36000	35000	32500	33250	860	10556
K^{+} (mg/l)	280	320	1500	1500	1270	1270	31	380
Cl^{-} (mg/l)	13614	13862	71155	69701	69148	69800	1540	18980
SO_4^{-2} (mg/l)	3983	1150	3100	2750	3200	4620	1236	2649
HCO_3 (mg/l)	248	145	122	134	110	122	207	140
TDS (mg/l)	29580	25407	126065	121150	120235	124360	4500	34483
Salinity (mg/l)	26385	23899	119926	117128	116282	118705	3766	35000
SAR	48.86	37.37	102.01	100.03	83.65	81.89	9.59	58.20

* Source: Major ion composition of sea water 1995-2009 and Millero et al., 2008.

Electrical conductivity (EC) is typically used to indicate soluble salt concentration and it is a rapid and convenient means of estimating the concentration of ions in solution. Since each ion has its own ability to conduct current, EC is only an estimate of the total ion concentration.

The EC values were measured for PW from the production facilities and disposal pits water, the results are shown in Table 3-2 and Figure 3-1. The EC for production facilities are 37057 and 38066 μScm^{-1} with an average 37561.5 μScm^{-1}, where the EC values during the disposal pit range from 187867 to 196977 μScm^{-1} with an average 192112 μScm^{-1}. The conductivity value of PW reached a maximum of 196977 μScm^{-1} at disposal pit in sampling point namely Spt1. This is considered to be very high, and indicates the presence of a high percentage of ionic compounds.

There was an increase in EC values in the PW samples collected from the pit compared to the samples taken from the production facilities over the study field Figure 3-1. This is due to evaporation and penetration through the soil column, which resulted in the accumulation of salts at the soil surface. This result was expected and agrees with other researcher's results such as Assadian et al., 2005 and Heidarpour et al., 2007.

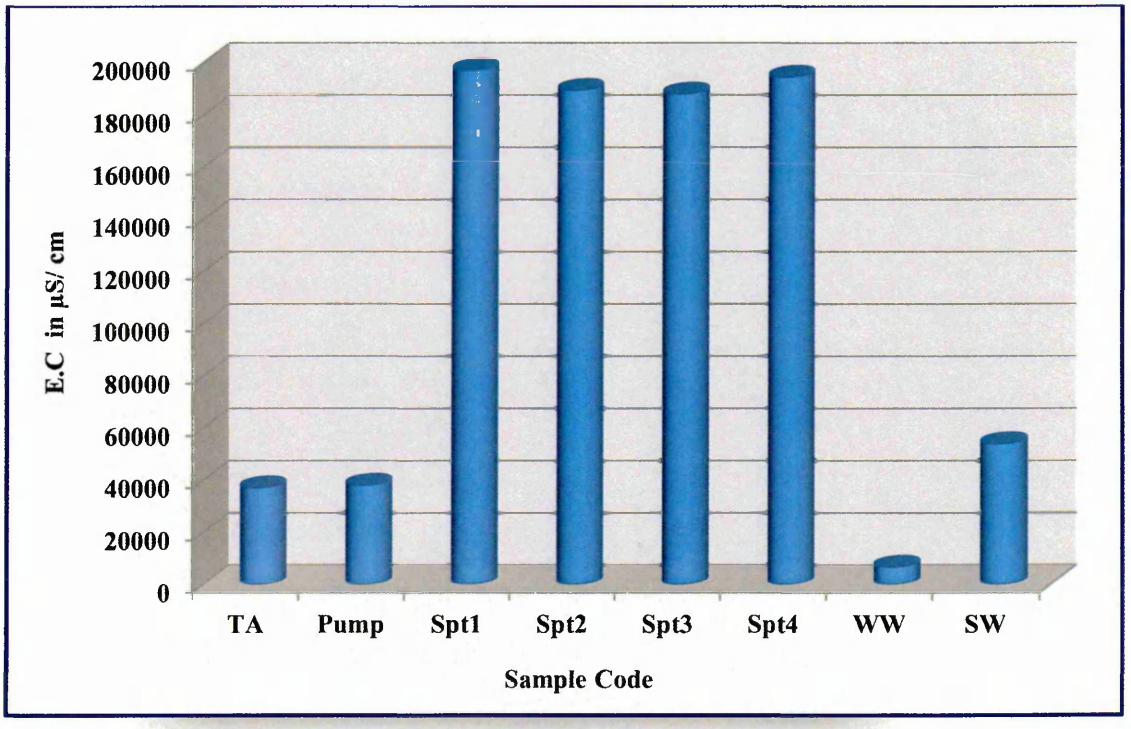


Figure 3-1 Electrical conductivity (EC) values for produced water at production facilities, pit bulk and ground water samples with comparing with sea water

The evaporation of water from the surface of a lake concentrates the dissolved solids in the remaining water and so it has a higher EC. Therefore, the disposal of PW at the surface in open environment causes high soil electrical conductivity (EC). The EC value for groundwater samples collected from water well close to the disposal pit shows a lower EC value than the PW, while the EC value for PW samples from bulk pits show a higher value than sea water as shown in Figure 3-1.

Total Dissolved Solid (TDS) is the sum total of all of the dissolved things in a given body of water. It includes hardness, alkalinity, chlorides, bromides, sulphates, silicates, and all manner of organic compounds and is an indication of all dissolved materials contained in PW. TDS values during the process are 25407 and 29580 mg/l, with an average of 27494 mg/l, while in the pit water bulk ranges from 120235 to 126065 mg/l, with an average of 122953 mg/l.

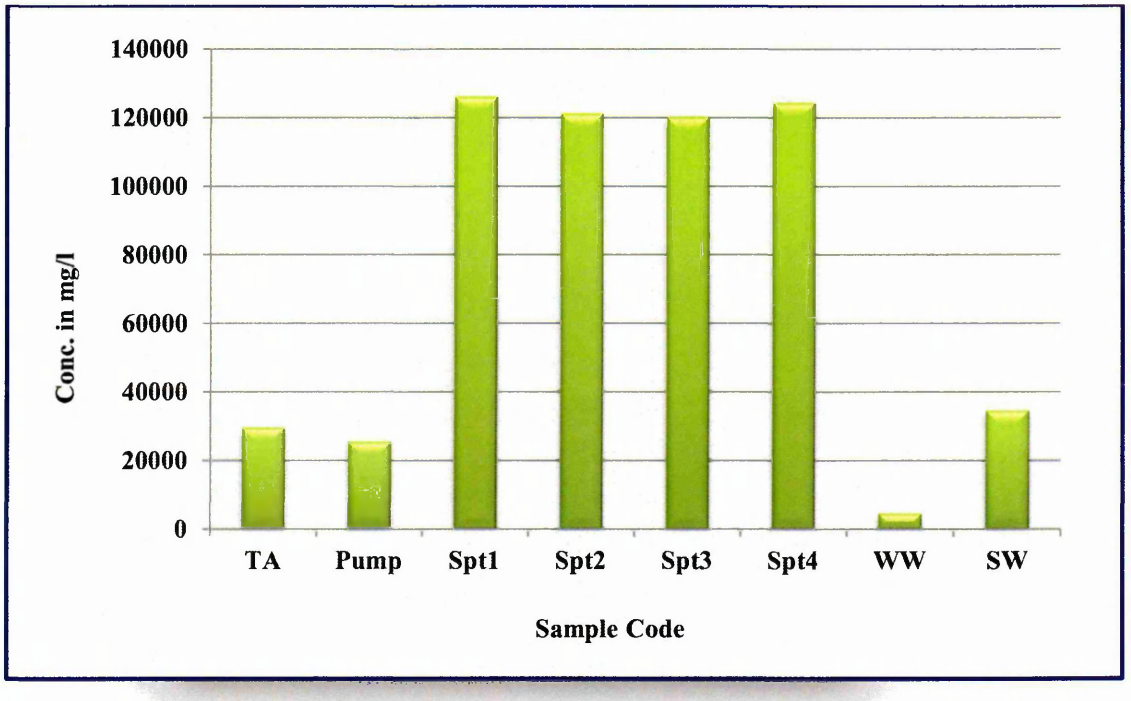


Figure 3-2 Total dissolved solid (mg/l) in produced water at production facilities, pit bulk and ground water samples with comparing with sea water

TDS concentration rises in bulk water pit; this is due to salt accumulation during the evaporation and penetration, the EC following the same trend as well. The groundwater has lower TDS concentration (4500 mg/l), while PW from the study site show higher TDS concentration than sea water (34483 mg/l) as show in Table 3-2 and Figure 3-2. Generally, oilfield waters contain much higher concentration of TDS than sea water does. PW has been reported with TDS concentrations ranging as little as 200 ppm to

saturation, which is about 300,000 ppm. Sea water contains about 35000 ppm of TDS (McCain 1990). EC of water is directly related to the concentration of dissolved ionized solids in the water. The relationship between TDS and EC is a function of the type and nature of the dissolved cations and anions in the water and possibly the nature of any suspended materials. The high EC and higher TDS were noted and these results give an evidence of high concentrations of cations and anions being carried by the PW. These are going to be accumulated in the soil over the long term of disposal. The composition of PW at the study area presented in Tables 3-2, show that the chloride concentrations range between 13614 to 71155 mg/l with an average of 51213 mg/l, (Chloride in seawater contains about 19,000 mg/l, (Holstad et al., 2005 and Johnson et al., 2008), while the chloride concentration in groundwater is much less than PW as show in Table 3-2. Chloride is an effective tracer for pollution from disposal of waste water or for salt water intrusion (Clesceri et al., 2005). Sulphate concentrations range between 1150 to 4620 mg/l with an average of 3134 mg/l, sulphate are present in high concentrations, Similarly, the main cations, sodium, potassium, calcium, and magnesium are found in high concentrations, Sodium concentrations range between 6650 to 36000 mg/l with an average of 25267 mg/l, Potassium concentrations range from 280 to 1500 mg/l with an average of 1023. Due to the large number of Na^+ ions available, the Na^+ ions are able to exchange with a sufficient number of the Ca^{2+} and Mg^{2+} ions, the Na^+ ion of sodium chloride causes the dispersion of the soil. The concentration of Ca and Mg in oilfield water is a function of the origin of the water, the ages of water and the enclosing rocks, and type of clay in the rock. Calcium concentrations range from 1348 to 8480 mg/l with an average of 5518 mg/l, magnesium concentrations range from 389 to 1993 mg/l with an average 1351 mg/l. In general the concentrations of the cation and anions in PW were higher than sea water, while in groundwater they are much lower than PW, as show in Table 3-2. The hardness of water is a measurement of the total concentration of the calcium and magnesium ions and expressed as calcium carbonate $CaCO_3$. Total hardness is a combination of general hardness and carbonate hardness. Total hardness in PW samples were measured and ranged from 5330 to 29400 mg/l with an average of 19355 mg/l. Calcium hardness and magnesium hardness as $CaCO_3$ (mg/l) were measured and it was observed that the concentrations of calcium hardness were significantly higher than magnesium hardness; the results are presented in appendix A. The physical and chemical properties of PW depend on geographic location of the oilfield and geological formations in contact with the water over time. The PW composition of the Nasser oilfield was compared with some other OPWs around the

world and is summarized in Table 3-3. The data shows that the ranges of pollutants present in the studied PW were less than or in the range of other PW studies in some areas around the world as shown in Table 3-3.

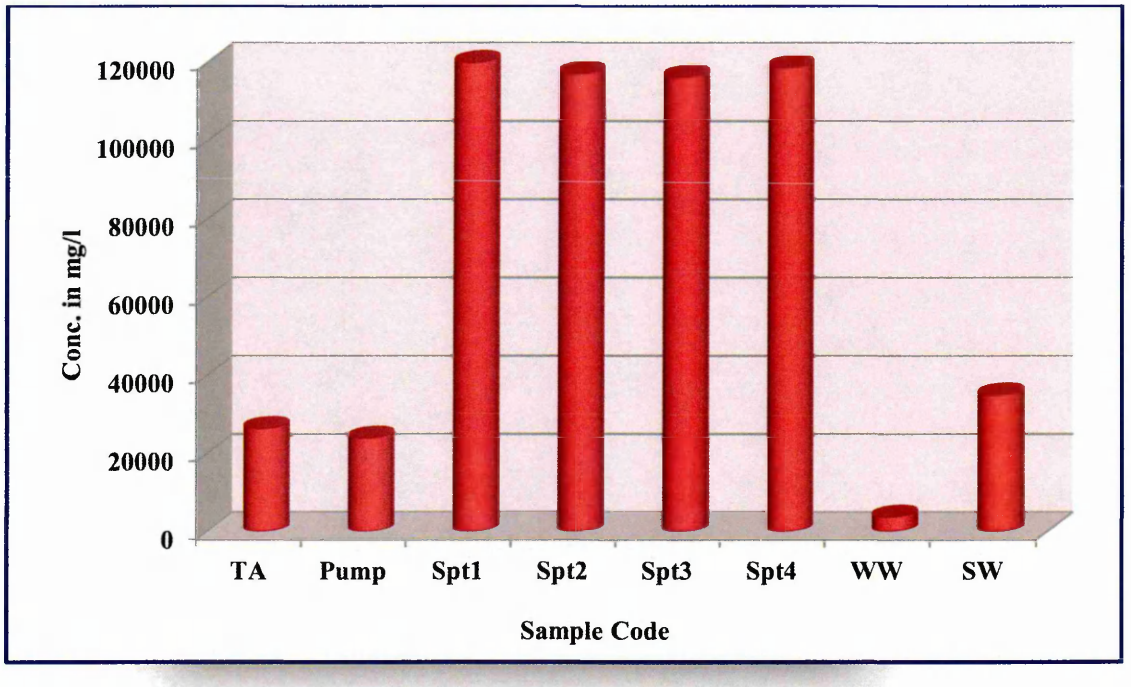


Figure 3-3 Salinity of produced water (PW) comparing with sea water (SW)

Salinity in water is usually reported as a lumped parameter, i.e. EC or TDS, total soluble cation (TSC) and total soluble anion (TSA). Salinity is a mix of electrolytes in which the charges of the cations and anions should be balanced assuming that all of the major ions have been analyzed (Lauchli et al., 2002). Salinity is one of the most significant environmental problems. It is important to identify saline areas so they can be appropriately managed. PW and groundwater salinities were measured and the results are presented in Table 3-2, the concentrations ranged between 23899 and 119926 mg/l with an average of 87054 mg/l. The results show that a high salinity was observed in PW at the pit, the water is more saline than sea water (35000 mg/l) and less salinity was observed in groundwater samples collected from the water well at the study site (3766 mg/l) as show in Figure 3-3. In general the chemical composition of groundwater samples obtained from water well at the Nasser Oilfield is much less than PW. High salinity levels in water cause significant problems for all users, agricultural, domestic and industrial.

Table 3-3 Summary of constituents (mg/l) in oilfield produced water in some areas around the world

Parameter	Egypt (Gulf of Suez)[a] (El-Said et al., 2009)	Saudi Arabia (Ghawar oilfield) Singh et al., 1996	UK (Wytch Farm oilfield) Worden et al., 2006	Mexico (Roswell oilfield) Tellez et al., 1995	USA (OSPER site Oklahoma) Kharaka et al., 2003 and Sirivedhin et al., 2004)	Libya (Gialo-59 oilfield) AbdolHamid et al., 2008	Libya (Nasser oilfield) (Producti Facilites
pH	7.17	7.10	-	6.8-7.2	6.1-6.8	6.41-7.16	6.67-6.9
EC (µS/cm)	93200	281719	-	78079.67	178125-318872	20773-61087	37057-380
TDS	62100	180300	-	49971	114000-185000	32458-95448	25407-295
Cl	37410	110500	111297-136306	25502	70100-113000	6235-27529	13614-138
HCO_3	376	210	4.90-119.30	-	105-280	220-1537	145-248
SO_4	153	255	1203-1645	621.3	0.23-0.81	1276-1864	1150-398
Mg	1732	1852	730-935	369.1	1530-2350	85-807	389-476
Ca	5361	16184	4455-5740	2668.9	5400-11900	60-1840	1348-176
Na	14682	46937	61520-81625	12878	24100-55000	5050-15000	6650-820
K	295	1747	2314-3450	-	100-690	150-370	280-320
Salinity as NaCl	61737	199563	181066-229532	46056.61	126601-204078	11260-49717	23899-263

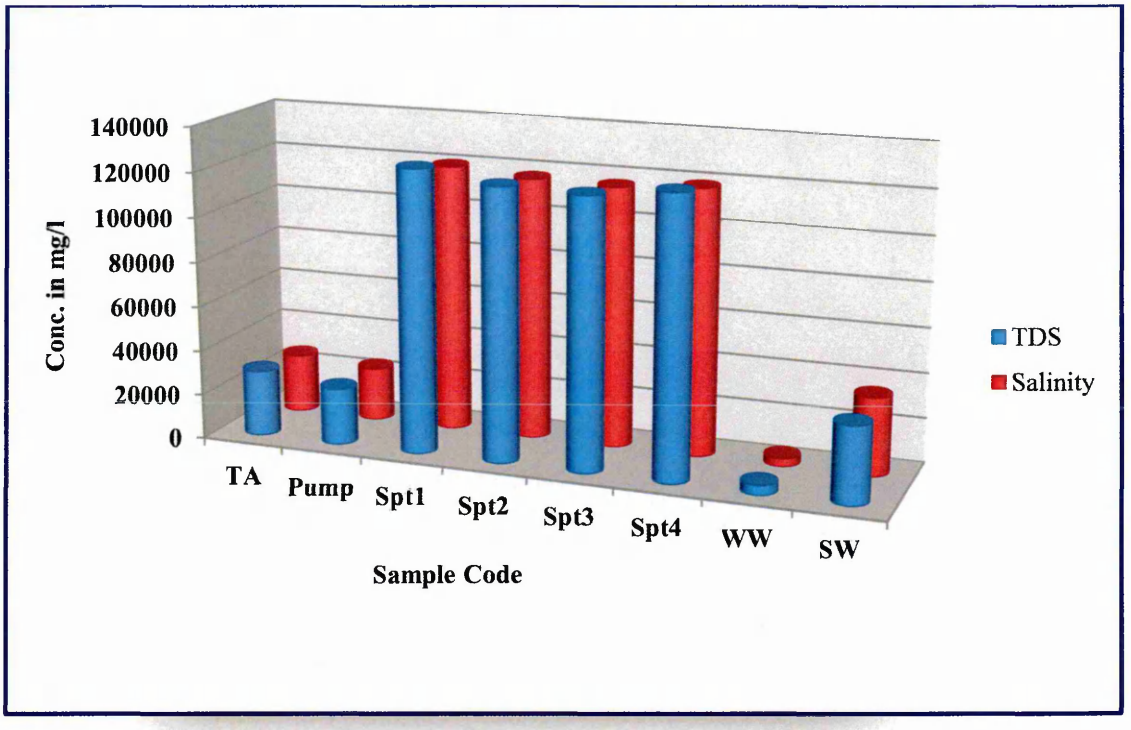


Figure 3-4 Relationship between concentrations of the salinity and total dissolved solid in produced water samples

The measurement of salinity was based on the measurement of TDS and the sum of soluble salts or the chloride ion concentration in the water or soil water extracts. The relationship between the salinity and TDS are plotted in Figure 3-4, it was observed that the PW from the pit bulk water has higher salinity and TDS than sea water, while the salinity and TDS was much less in groundwater samples collected from ground water well at the study site.

The chemical composition of the ground water well located in the North part of the disposal pit in the study has lower values for salinity, TDS, Na, Mg and Ca as well as for Cl when compared with PW as shows in Figures 3-3 and 3-4. These chemical properties that are different for produced and ground waters are used to investigate the impact of PW on the surface and ground waters of the contaminated areas. SAR is a ratio of the sodium to the combination of calcium and magnesium in relation to known effects on soil dispersibility, another critical parameter for irrigation water. The SAR results for PW range between 37.37 to 102.01 meq/l with an average of 75.63. These are much higher than the SAR for sea water as shows in Figure 3-5. The SAR for groundwater was 9.59 meq/l which is much less than the PW and within the standards for water. According to (Sirivedhin et al., 2004) high SAR values will decrease the movement of water through the soil which means that the soil becomes less permeable,

and causes the death of plants. SAR is crop and soil-dependent and is also affected by salinity. Water used in irrigation a with high SAR (> 6) will indicate cation exchange processes on clay particles with Na replacing K, Ca and Mg resulting in decreased in soil permeability (Horner et al., 2011).

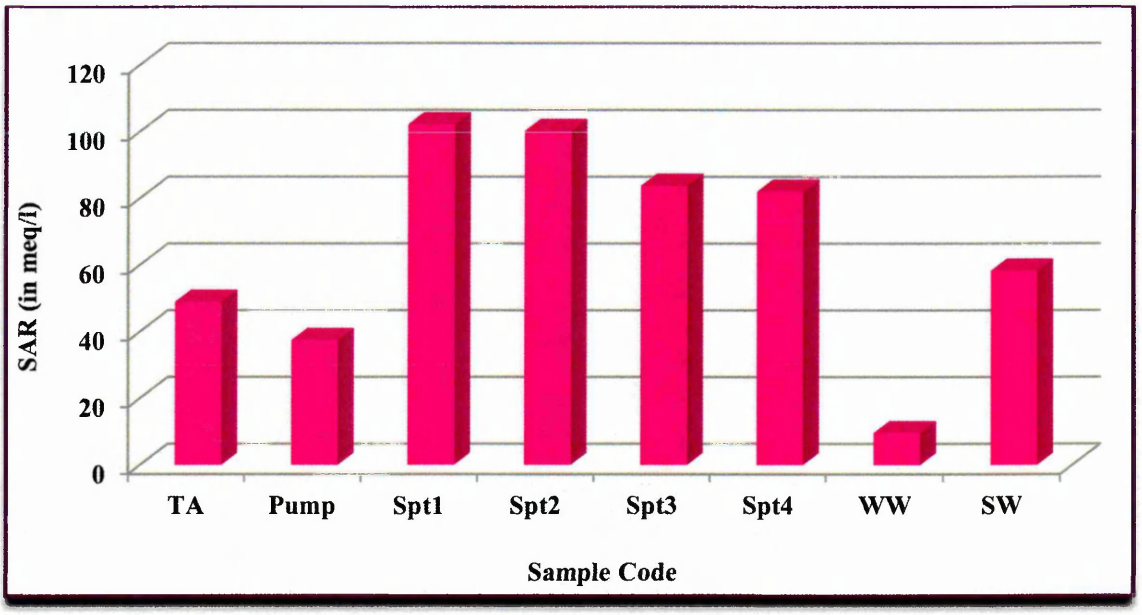


Figure 3-5 Sodium adsorption ratios (meq/l) for produced water and groundwater samples from the Nasser oilfield with comparison with sea water

Exploration and production waste from oilfields such as PW usually contains low quantities of heavy metals and some of these trace elements occur naturally in water and are required in low concentrations. However, some trace elements in PW can cause harmful effects to plants when present in sufficient quantities.

Trace element concentrations of PW and groundwater samples collected from the site are summarized in Table 3-4. The data show that PW contains high concentrations of dissolved strontium, barium and manganese. Strontium concentrations ranged from 30.99 mg/l to 158.30 mg/l, barium concentrations ranged between 0.246 mg/l to 0.578 mg/l and manganese concentrations ranged between 0.105 mg/l to 0.290 mg/l., while in groundwater these are much lower than produced water as show in Table 3-4. Arsenic, copper, iron, nickel, lead, and zinc are detected in traces. Cadmium, cobalt, chromium, mercury and selenium are found in very low concentrations or not detected. The not detected results would suggest that a more sensitive technique for determination of heavy metals would be preferred. Nevertheless, it is evident that heavy metal concentrations can exceed levels at which toxic effects would be expected. Trace element concentrations in the PW collected from pit bulk are higher than those at the

processing point due to evaporation and accumulation of these elements in the pit. The same trace elements in groundwater were either not detected or in very low concentrations, much less than the PW as shown in Table 3-4.

Table 3-4 Concentrations of trace metals in PW using ICP technique

Sample Code / Element (mg/l)	TA	Pump	Spt1	Spt2	Spt3	Spt4	WW
As	< 0.02	0.024	0.071	< 0.02	0.146	0.035	< 0.02
Ba	0.246	0.336	0.563	0.493	0.502	0.578	< 0.07
Cd	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Co	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Cr	< 0.002	< 0.002	0.003	< 0.002	< 0.002	0.004	< 0.002
Cu	0.030	0.030	0.032	0.030	0.029	0.027	< 0.07
Fe	0.054	0.054	0.105	0.066	0.055	0.060	< 0.01
Hg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mn	0.105	0.160	0.290	0.088	0.233	0.151	0.032
Ni	< 0.001	< 0.001	0.01	0.008	0.006	0.008	< 0.001
Pb	< 0.03	< 0.03	0.05	0.08	0.08	0.07	< 0.03
Se	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
Sr	30.99	38.24	154.40	128.40	135.20	158.30	< 0.07
Zn	< 0.01	< 0.01	< 0.01	0.08	0.1	0.1	< 0.01

3.5.2 Soil samples

The soil is considered the main affected media since it is in direct contact with the PW. The impacts on the soil of the site were identified by measuring the physical and chemical properties of eighteen polluted soil samples and one uncontaminated soil sample. The physical and chemical properties of the studied soils are presented in Tables 3-5, 3-6 and 3-7. The results from the analysis of soil for their physical and chemical properties indicate that: pH values for polluted soil samples ranged between 6.06 to 7.95, with an average of 7.17. These are within the normal range in comparison to the soil reference sample (pH 7.30), as shown in Tables 3-5 to 3-7. Electrical Conductivity, (EC) values, for polluted soil samples were high, and ranged between 31868 to 161080 μScm^{-1} with an average 72830 μScm^{-1}. The EC for the reference soil

sample was 2346 μScm^{-1}, which is considered low and within the characteristics of the ground at the ambient area, as it depicted in Figure 3-6 and Tables 3-5 to 3-7. Similarly, TDS values for polluted soil samples were high, which ranged from 20716 to 105240 mg/kg with an average 46482 mg/kg, while in reference samples, TDS was 1535 mg/kg, which is within normal characteristics, as it is listed in Tables 3-5 to 3-7 and plotted in Figure 3-7. The high values of EC and TDS for contaminated soil are referring to the high content of salts which are accumulated at the soil due to direct contact with disposal of PW at the surface soil at the study site. The full chemical and physical composition of contaminated soil and uncontaminated soil samples collected from the study area is presented in appendix B.

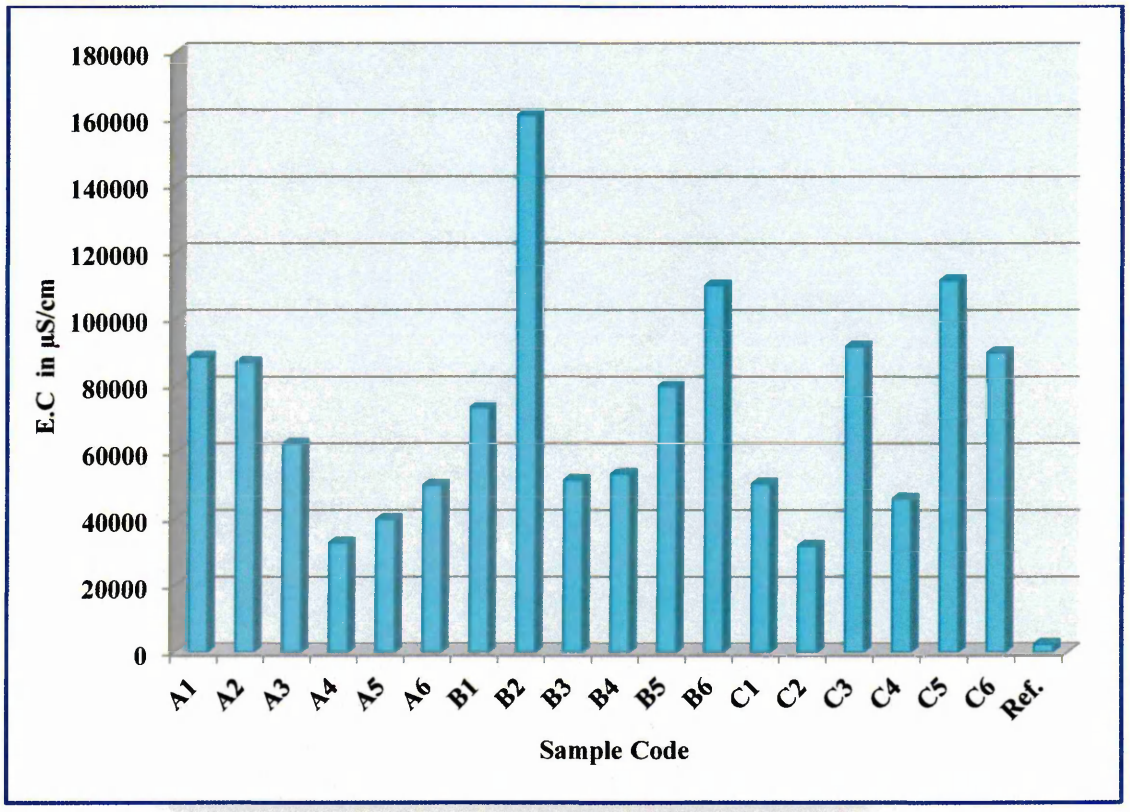


Figure 3-6 Electrical conductivity values for soil sample

Table 3-5 Chemical and physical characteristics of polluted soil samples

Parameter	A1	A2	A3	A4	A5	A6
pH	7.23	7.92	7.11	7.19	7.32	7.42
EC (μScm^{-1})	88543	86826	62434	32800	39872	50036
Ca^{+2} (mg/l)	2336	2720	2496	2040	2196	1940
Mg^{+2} (mg/l)	1380	1070	574	278	538	490
Na^{+} (mg/l)	17000	15000	10800	4760	5800	8000
K^{+} (mg/l)	360	580	280	200	230	250
Cl^{-} (mg/l)	31693	27398	20376	8740	11708	15694
SO_4^{-2} (mg/l)	4048	5600	3518	4300	3956	2326
HCO_3 (mg/l)	61	48	146	136	104	98
TDS (mg/l)	57525	54700	39640	21300	25840	29960
Salinity (mg/l)	56073	50514	36744	18380	23136	27956
SAR	68.91	61.67	50.68	26.21	28.77	42.03

Table 3-6 Chemical and physical characteristics of polluted soil samples

Parameter	B1	B2	B3	B4	B5	B6
pH	7.95	6.06	7.24	7.00	7.38	6.65
EC (μScm^{-1})	73322	161080	51586	53400	79746	110000
Ca^{+2} (mg/l)	2560	4096	2448	2520	3040	2000
Mg^{+2} (mg/l)	874	2370	1142	680	874	550
Na^{+} (mg/l)	11500	32000	7600	8600	13000	23200
K^{+} (mg/l)	720	640	330	336	360	400
Cl^{-} (mg/l)	19310	62722	18188	16798	22440	38358
SO_4^{-2} (mg/l)	8400	1880	1950	4800	8000	3800
HCO_3 (mg/l)	48	86	146	146	122	136
TDS (mg/l)	43412	105240	32900	33300	50240	72018
Salinity (mg/l)	39922	104960	31742	31638	44468	66894
SAR	50.17	98.51	31.80	39.25	53.50	118.44

Table 3-7 Chemical and physical characteristics of polluted soil samples

Parameter	C1	C2	C3	C4	C5	C6	Ref.S
pH	7.47	7.60	6.62	6.85	7.48	6.61	7.27
EC (μScm^{-1})	50524	31868	91586	46000	111484	89824	2346
Ca^{+2} (mg/l)	2820	1960	4484	2080	4000	3340	152
Mg^{+2} (mg/l)	598	1094	2554	462	972	1398	44
Na^{+} (mg/l)	8200	3500	12400	7600	20250	16000	280
K^{+} (mg/l)	320	190	580	250	680	440	18
Cl^{-} (mg/l)	18086	8884	26506	13610	39211	33008	551
SO_4^{-2} (mg/l)	2122	4532	11576	4400	3550	2720	360
HCO_3 (mg/l)	158	170	134	220	134	184	73
TDS (mg/l)	32840	20716	59532	27920	71350	58244	1535
Salinity (mg/l)	31584	18936	54678	26902	67678	56826	1281
SAR	36.58	15.71	36.62	39.25	75.20	58.63	5.14

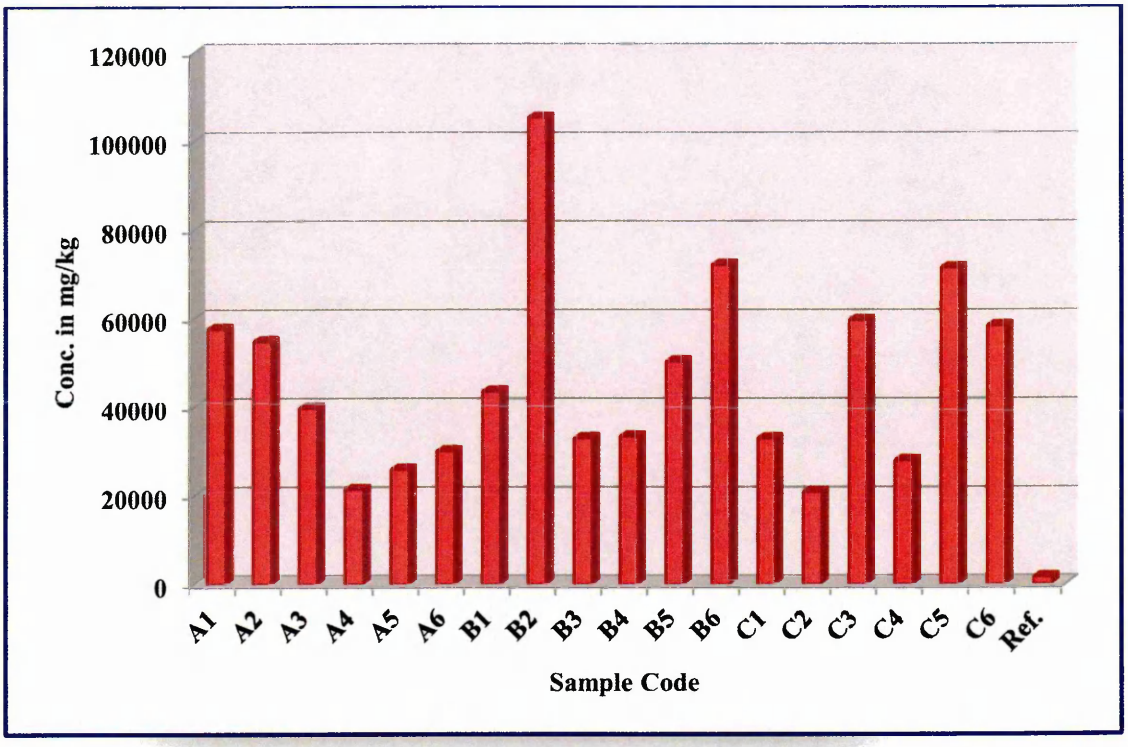


Figure 3-7 Concentration of total dissolved solid in soil sample of the study area.

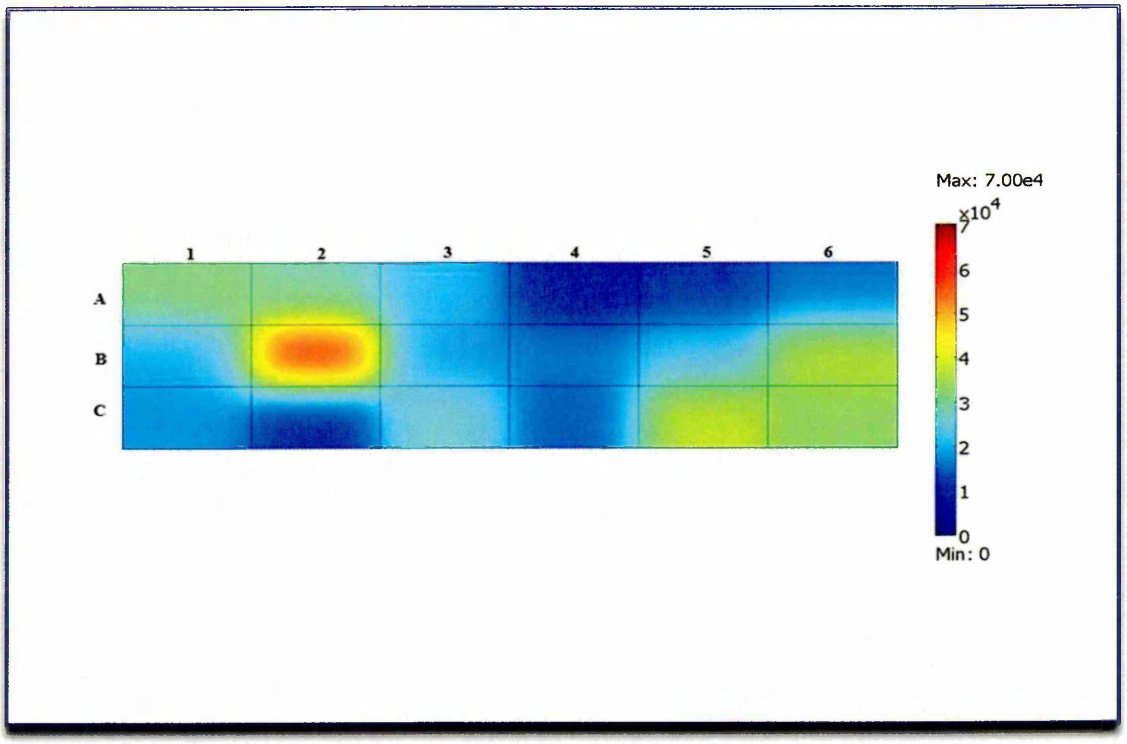


Figure 3-8 Chlorides distribution profile through the surface soil at the study area

The analyses of soil samples for anions show that the chloride concentrations ranged between 8740 and 62722 mg/kg with an average of 24041 mg/kg, sulphate concentrations ranged between 1880 and 11576 mg/kg with an average of 4082 mg/kg, and bicarbonate concentrations ranged from 48 to 220 mg/kg with an average of 126.5 mg/kg. These are considered to be high when compared to the reference soil sample (Na^+: 551 mg/kg, SO_4^{2-}: 360 mg/kg and HCO_3^-: 73 mg/kg). Figure 3-8 shows the distribution profile of chlorides in the surface soil at the study area. The analysis of soil samples for cations indicated that the concentrations of sodium ranged between 3500 and 32000 mg/kg with an average of 12512 mg/kg. Potassium concentrations ranged between 190 and 720 mg/kg with an average of 397 mg/kg. Calcium concentration ranged between 1940 and 4484 mg/kg with an average of 2626.4 mg/kg and magnesium concentration ranged between 278 and 2554 mg/kg with an average of 994.3 mg/kg. In the reference soil sample the concentrations of the cations can be considered to be normal; sodium 280 mg/kg, potassium 18 mg/kg, calcium 152 mg/kg and magnesium 44 mg/kg. High Na concentration in the soil may cause clay particle swelling and dispersion, resulting in the deterioration of soil physical conductivity (Rashidi et al., 2008 and Chhabra 1996). Increasing Ca and Mg content would lead to a potential decrease in Na damage or influence on soil (Heidarpour et al., 2007).

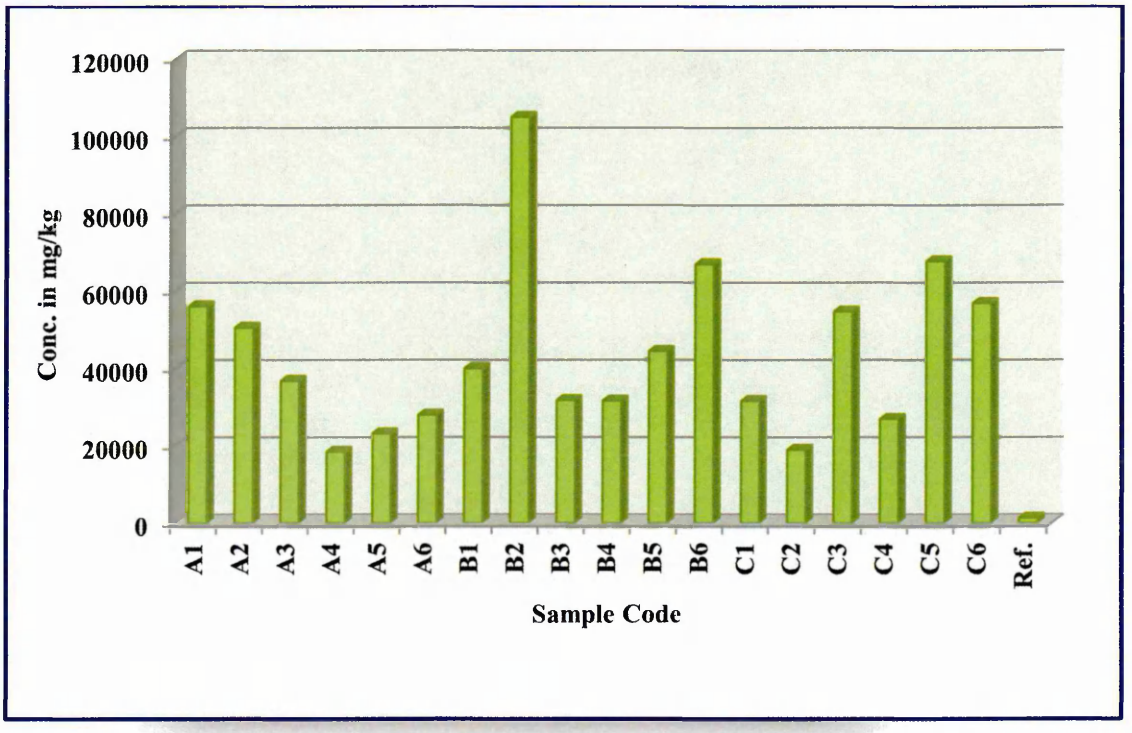


Figure 3-9 Salinity values for soil sample collected from the study area

Salinity becomes a concern when an "excessive" amount or concentration of soluble salts occurs in the soil, either naturally or as a result of contamination. The salinity of contaminated and uncontaminated soil samples was measured and the results are presented in Tables 3-5 to 3-7 and Figure 3-9. The soil obtained from the Nasser oilfield has a salinity range between 18380 and 104960 mg/kg with an average of 43837 mg/kg. This is considered to be very high compared to the reference sample (1281 mg/kg) as shows in Figure 3-9. These results are comparable to that of the PW. The high salinity in soil was expected since the PW has elevated levels of TDS associated with as shown in Figure 3-10 and this will be accumulate on the surface of soil as shown in Figure 3-11. Soil with high salinity levels causes significant problems for all users, agricultural, domestic and industrial. High salinity has an adverse effect on crop growth by increasing osmotic pressure which makes the water in the soil less available for plants. In addition the salt accumulating on the top soil will reduce the infiltration rates of water into the soil (Qadir et al., 2004).

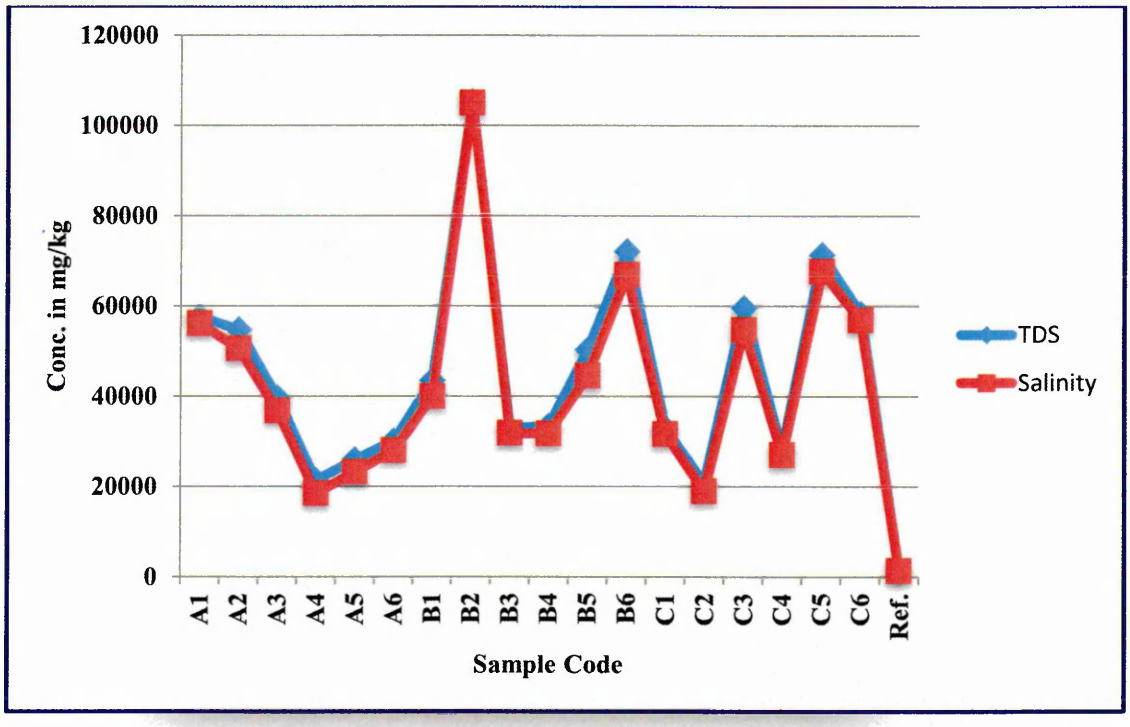


Figure 3-10 Relation between salinity and TDS concentrations in soil at the site

Figure 3-11 Salts accumulated on top soil at the pit bank of the study area

Sodium levels in soil are often reported as the SAR, a ratio of the amount of cationic (positive) charge contributed to a soil by sodium, to that contributed by calcium and magnesium, When the SAR is high, this will cause soil structure deterioration and water infiltration problems (Chhabra 1996). According to Davis et al., 2010, when sodic soils are wet, they become sticky, and when dry, they form a crusty, nearly impermeable layer and clearly seen at the studied site as shown in Figure 3-11. The SAR results for soil samples ranged between 15.71 and 118.44 meq/l with an average of 50.72 meq/l, which is high with compared with the reference soil sample (5.14 meq/l) as shows in Figure 3-12. According to Chhabra 1996 and Davis et al., 2010, SAR values above 13 lead to soil dispersion and loss of soil infiltration capability.

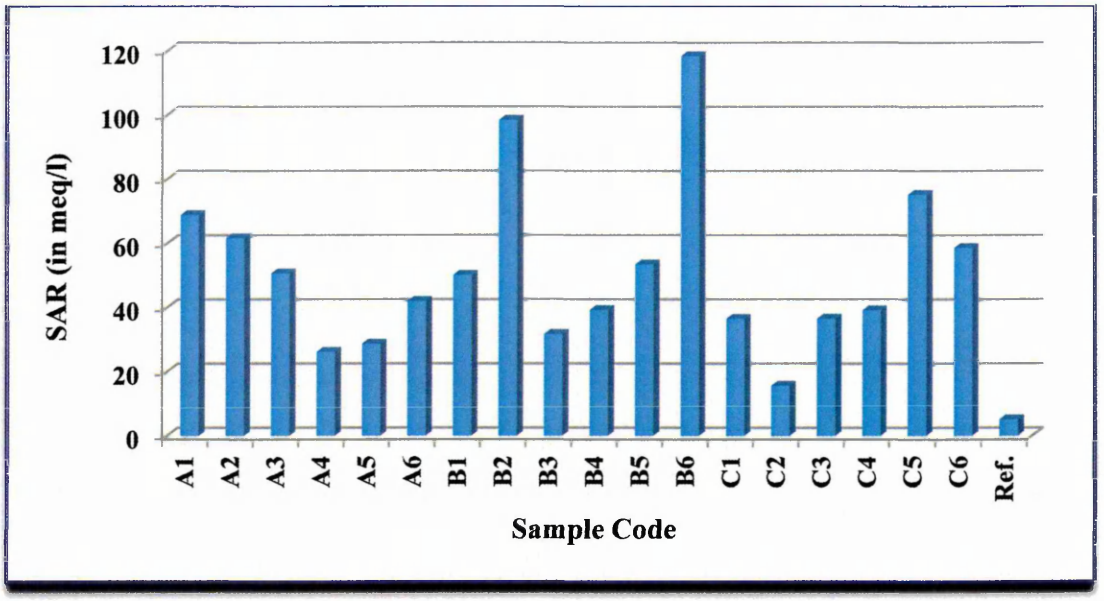


Figure 3-12 Sodium adsorption ratios (meq/l) for soil from the Nasser oilfield

Several trace elements occurring naturally in soil are required in low concentrations. Trace elements can be introduced into soil through a variety of sources. The trace element concentrations of the soil samples collected from the edge of the disposal pit are summarized in Tables 3-8 and 3-9. Results shows that trace elements concentrations that have been found in polluted soil vary, Fe between 1056 mg/kg and 3282 mg/kg, Sr between 93.27 mg/kg and 204.70 mg/kg, Mn between 36.11 mg/kg and 140.0 mg/kg, Ba between 16.60 mg/kg and 42.32 mg/kg, Zn between 1.77 mg/kg and 107.40 mg/kg, Cr between 5.99 mg/kg and 24.67 mg/kg, Cu between 1.76 mg/kg and 3.99 mg/kg, Ni between < 0.01 to 11.56 mg/kg, Pb between < 0.03 to 12.39 mg/kg, Pb between Se between < 0.04 to 6.35 mg/kg, As between < 0.02 to 4.98 mg/kg and Co between < 0.004 to 3.25 mg/kg while the concentrations of the above trace elements in reference soil sample are 965 mg/kg, 80.53 mg/kg, 24.64 mg/kg, 8.07 mg/kg, 3.90 mg/kg, 7.61 mg/kg, 2.03 mg/kg, < 0.01 mg/kg, < 0.03 mg/kg, < 0.04 mg/kg, < 0.02 mg/kg and 1.27 mg/kg respectively. The results obtained for uncontaminated soil taken far from the contamination zone where used for the comparison, indicates that the soil was contaminated to varying degree, except for Cd and Hg which are close to the reference soil samples as shown in Tables 3-8 and 3-9.

Table 3-8 Concentrations of various metals in soil sample as found by ICP

Sample Code / Element (mg/kg)	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4
As	< 0.02	< 0.02	< 0.02	4.98	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Ba	36.42	32.06	18.77	24.98	24.56	33.03	20.43	39.46	31.87	29.47
Cd	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Co	< 0.004	1.07	0.13	< 0.005	2.25	2.60	0.45	1.54	1.13	< 0.004
Cr	17.35	7.90	11.51	24.67	9.58	16.74	5.99	12.96	21.28	14.63
Cu	3.99	1.98	2.62	2.44	2.47	3.44	1.76	3.35	2.59	3.76
Fe	2070	2861	1451	1695	1207	1322	1550	2176	2622	1790
Hg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mn	63.70	38.52	36.40	47.78	45.53	52.58	36.11	79.91	81.45	56.97
Ni	8.02	3.67	6.10	8.40	5.48	8.84	0.61	7.28	6.90	< 0.01
Pb	9.60	< 0.03	< 0.03	< 0.03	3.65	< 0.03	< 0.03	< 0.03	6.88	< 0.03
Se	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	4.08	< 0.04	5.22	2.89	< 0.04
Sr	166.80	192.60	85.05	119.90	101.20	111.00	129.00	160.50	176.80	125.9
Zn	25	107.40	15.61	25.22	14.57	10.18	48.11	23.21	50.27	7.35

Table 3-9 Concentrations of various metals in soil sample as found by ICP

Sample Code / Element (mg/kg)	B5	B6	C1	C2	C3	C4	C5	C6	Re.S
As	< 0.02	< 0.02	< 0.02	3.03	< 0.02	1.70	< 0.02	2.04	< 0.02
Ba	19.69	22.46	19.58	28.10	41.32	16.60	26.05	31.77	8.07
Cd	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Co	0.69	< 0.005	2.385	3.25	0.30	1.58	0.82	1.01	1.27
Cr	14.90	18.36	14.97	20.58	6.02	13.90	11.00	21.69	7.61
Cu	3.37	2.39	3.78	2.99	2.422	1.91	2.67	2.96	2.03
Fe	1695	1933	1593	3282	2178	1250	1056	2717	965
Hg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Mn	47.07	47.11	140.00	89.79	133.30	39.33	39.67	78.96	24.64
Ni	2.87	8.06	< 0.01	9.70	< 0.01	1.47	10.32	11.56	< 0.01
Pb	12.39	< 0.03	2.46	< 0.03	< 0.03	< 0.03	< 0.03	11.68	< 0.03
Se	1.24	6.25	< 0.04	1.82	< 0.04	< 0.04	< 0.04	6.35	< 0.04
Sr	118.40	146.90	158.90	154.00	204.70	93.27	94.73	198.00	80.53
Zn	10.16	16.31	18.74	7.43	11.28	1.77	4.35	33.37	3.90

3.6 Conclusions

Conclusions can be drawn from the results of the measurement of the physical and chemical properties of PW generated by the Nasser oilfield operation; the high saline produced water is considered the major carrier of cations and anions and impact on the surrounding environment, where these accumulated materials (cations and anions) interrupt the environmental equilibrium and change the physical properties of the soil (permeability, porosity, hydro-conductivity) and may affect the bio-organisms present in the soil which are considered as elements of the ecosystem.

Unfortunately, under influence of PW discharges from the Nasser Oilfield operation at the surface soil the changes in properties of soil are likely. Soil samples obtained from the disposal pit at the study site show variable impacts from the disposal of PW on the surface. This soil was contaminated by saline water over long term of disposed: so high concentration of anions and cations exist in the soil, especially sodium, magnesium, calcium, potassium, chloride and sulphate. These cause the poor physical condition of the soil and according to the results the soil is considered as saline and sodic. It was observed that the soil was suffering from high concentration of salts. Where the analysis results of reference soil samples taken from 250 meter from the edge of the pit are uncontaminated and were employed to make a comparison with the results of polluted samples. The result of the soil samples shows that the extent of pollution or influenced in soil was extent up to 20-25 meters from the edge of the pit.

Chapter Four
Hydrocarbon analysis (TPH, BTEX and PAH) in soil and produced water

4.0 Introduction

As described in the introductory chapter, crude oil is a naturally occurring liquid consisting of a complex mixture of a wide range of hydrocarbon and non-hydrocarbon compounds of different molecular weights. Crude oil from different sources around the world shows a wide range of physical and chemical properties (Mills et al., 1999 and Kok 2011). Pollution arising from crude oil production is of great concern for the environment and has been so for more than two decades with little solution to this problem due to increasing reliance on oil products around the world. Pollution caused by crude oil and its petroleum products is the most common problem with the release of the crude oil into the environment receiving worldwide attention. Petroleum hydrocarbons are toxic and have adverse affects on human health and to the environment (Villalobos et al., 2008, Millioli et al., 2009, Deriszadeh et. al., 2010, Atagana 2011, and Basumatary et al., 2012). The oil and gas production industry generates large volume of PW which is brought up to the surface with crude oil. Basically, PW is a combination of formation and injection waters that contains oil, salts, solids, trace elements and OFCs. PW is the primary waste product resulting from the separation of crude oil and natural gas at the production facilities (Lu et al., 2006 and Ebrahimi et al., 2012), PW as discussed previously usually contain organic constituents such as dispersed droplets of crude oil, dissolved hydrocarbon (volatile hydrocarbon BTEX and aromatic hydrocarbon PAH). These hydrocarbons in PW can be found with varying concentrations as free floating, emulsified, dissolved, or adsorbed to suspended solids which are discharged into the environment associated with the PW (Thomas et al., 2004 and Lu et al., 2006).

A hydrocarbon, by definition, is one of a group of chemical compounds composed only of hydrogen and carbon. However, the most harmful contaminants present in PW are benzene, toluene, ethylbenzene, and xylene (BTEX), polyaromatic hydrocarbons (PAHs), phenols and heavy metals (Ranck et al., 2005 and Tellez et al., 2005). Research on the fate and transport of oil in the environment requires analytical techniques that can provide more detailed information on the components of petroleum hydrocarbons than traditional standard methods. Therefore, total petroleum hydrocarbons (TPH) determination is the initial and most general indicator of hazards posed by hydrocarbons for environment. This PW has to be analyzed with relation to the chemical composition to deduce the environmental impact of its discharge. Therefore, a study was carried out to evaluate preliminarily the TPH, BTEX and PAHs contents in PW samples taken from the production facilities and disposal pit at the Nasser oilfield, Libya. Several methods

for the measurement of hydrocarbons (TPH, BTEX and PAH) have been established for water, sediment, and soil samples to give information on petroleum in the environment such as gravimetric, infrared spectroscopy and gas chromatography methods (Mills et al., 1999 and Villalobos et al., 2008).

This chapter will study and evaluate the concentrations of TPH, BTEX and PAHs in PW and impacted soil samples taken from the Nasser Oilfield, where the bank of disposed pit has been chosen as a case study area (Figures 2-17 and 2-18). Different methods were optimized to determine the target compounds. PAHs were determined by gas chromatography with mass spectrometry (GC/MS) volatile aromatic hydrocarbons (BTEX) by gas chromatography with flame ionization detector (GC/FID) and TPHs were measurement by using gravimetric, IR and gas chromatography methods. Furthermore the distribution of the TPH at the soil caused by the disposed of the oily PW and its impact at the oilfield of the case study were investigated.

4.1 Materials and methods

4.1.1 Materials

All solvents were Chromasolv for HPLC grade ≥ 99.9%. Dichloromethane (boiling point, 39.8-40°C, density, 1.325 g/ml at 25°C), hexane (boiling point, 69.0°C, density, 0.66 g/ml at 25°C). Methanol (boiling point, 64.8°C, density, 0.791 g/ml at 25°C) and acetone (boiling point, 56.0°C, density, 0.791 g/ml at 25°C) were obtained from Sigma Aldrich, UK, polychlorotrifluoroethylene S-316 (boiling point, 134°C, density, 1.75 at 25°C) was obtained from Horiba, Ltd, Japan, hydrochloric acid (HCl) was of analytical grade purchased from Sigma Aldrich, UK, sodium sulpahte was of analytical grade purchased from Fisher Scientific, UK, silica gel 70-230 mesh with pore size 60A was obtained from Sigma Aldrich, UK. C18 cartridges (500mg/3ml) were purchased from Supelco, UK. All water used was Milli-Q grade.

The purity of the solvents used for the extraction and preparation of standards were checked before use. Mixed standards of TPH, BTEX and PAHs with concentrations of 2000 µg/ml were purchased from Supelco, UK, with purities of 95 % - 99 % were used and prepared in suitable solvents and stored at 4°C in the dark. The PW and impacted soil samples used in this study were collected from the Nasser Oilfield, Libya where the bank of the disposal pit has been chosen as a case study area as shown in Chapter two (Figures 2-17 and 2-18).

4.1.2 Methods

Determination of hydrocarbon in polluted sample is one of the most commonly performed analyses in the study of any site contaminated with petroleum hydrocarbon.

Due to the wide range of hydrocarbon contaminants that can potentially enter the environment, a suitable method to measure the concentration of pollutants is needed (Villalobos et al., 2008). Various organizations such as the American Society for Testing and Materials (ASTM), Environmental Protection Agency (EPA) have developed standard methods for the determination of petroleum hydrocarbons. A number of analytical techniques are available for measuring low, high molecular weight and TPH in samples of water, soil and sediment. The most popular TPH methods are based on gravimetric, gas chromatography (GC) and infrared spectroscopy (IR) analytical techniques (Wang et al., 1997; Villalobos et al., 2008). Environmental samples are generally not suitable for direct analysis as they are either too dilute (water) or too complex (soil and sediment). In this case sample preparation e.g. extraction is a very important step. A number of extraction techniques (Figure 4-1) are available such as soxhlet extraction (SE), automated soxhlet extraction (ASE), liquid-liquid extraction (LLE), solid phase extraction (SPE), ultrasonic extraction (USE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and microwave assisted extraction (MAE). These techniques have been developed to improve some important criteria such as efficiency of the extraction technique, reducing the sample preparation time, reducing the solvent consumption (Mitra 2003). Extraction means methods used to separate out various components from a matrix using a solvent or is the process in which solutes desorb from the sample matrix and then dissolve into the solvent. It is commonly used for liquids and solids. There are three interrelated factors influencing the extraction efficiency solubility, mass transfer and matrix effects (Mitra 2003). The choice of the solvent is an important step in the extraction procedure and is dependent on the analyte or analyte-mix. The samples clean-up steps were simplified, because of the cleaner extracts provided by modern extraction techniques.

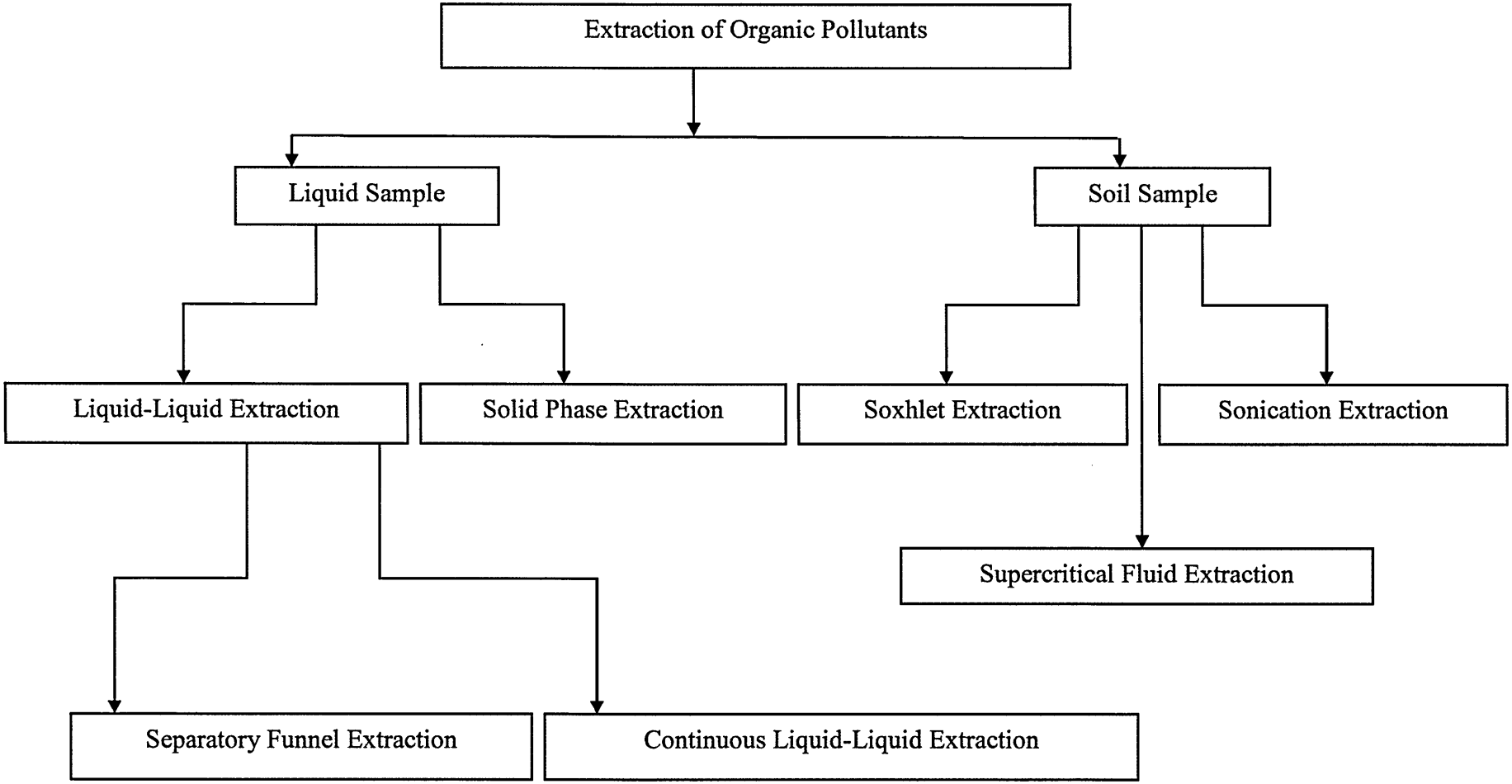


Figure 4-1 Schematic diagram of various extraction methods

4.2 Analytical methods for the determination of petroleum hydrocarbons in soil and produced water samples

Petroleum hydrocarbons are a mixture of various substances containing carbon and hydrogen. Petroleum hydrocarbons are important environmental contaminants and the determination of petroleum-derived hydrocarbons is important. Petroleum hydrocarbons are toxic and have adverse affects on human health and the environment (Villalobos et al., 2008, Park et al., 2011 and Malik et al., 2012). Three common analytical methods have been used in this study for the measurement of TPH in soil and PW samples, gravimetric, gas chromatography with flame ionization detection (GC-FID) and the use of infrared spectroscopy (TPH-IR). EPA approved and published methods for the measurement of TPH in soil and PW samples were used in this investigation. The following analytical equipment and standard test methods are used for the measurement of petroleum hydrocarbons in soil and PW samples

4.2.1 Determination of total extractable hydrocarbon (TEH) in soil and produced water samples using a gravimetric method

A gravimetric method is a method used for the quantitative determination of an analyte based on the mass of a solid. Gravimetric methods mesure any material extracted by a solvent and not removed during solvent evaporation, and capable of being weighed. A gravimetric method is very simple, quick, and inexpensive. Detection limits are approximately 5-10 mg/l in water and 50 mg/kg in soils (Weisman 1998; Villalobos et al., 2008). Gravimetric methods are not suitable for measurement of light hydrocarbons and can be used for heavy hydrocarbons, so they are recommended for TPH measurement only for very oily sludge or for samples containing heavy molecular weight hydrocarbons. The removal of hydrocarbon from soil samples using continuous extraction with a solvent has been done for many years by the soxhlet extraction method. Gravimetric methods give no information on the type of hydrocarbon present, no information about the presence or absence of toxic compounds, and no specific information about potential risk associated with the contamination (Wang et al., 1997, Weisman 1998 and Villalobos et al., 2008).

4.2.1.1 Soil sample preparation

20 g of soil samples (W1) was mixed with 10 grams of sodium sulphate anhydrous and placed in an extraction thimble and extracted using 30 ml DCM in an auto-soxhlet system as shown in Figure 4-2. The auto-soxhlet system conditions used were as follows: solvent used DCM, temperature 130°C, immersion time 25 min, washing time

120 min and solvent recovery 15 min. The apparatus is constructed so that the solvent was heated; the vapour rises, cools, condenses and falls into the thimble. The thimble fills and a draw off tube removes the solvent taking it back to the boiling solvent and the process is repeated continuously for 120 min. The extracts were dried by passing them through a drying column containing about 10 grams of anhydrous sodium sulphate then the solvent was complete evaporated (Venosa et al., 1996, Li et al., 2005 and EPA method 3550C, 2007). The residues were dried and weighed (W2) and the concentration of total extractable hydrocarbon (TEH) or DCM- extractable organic materials was calculated (EPA method 9071B 1998) as follows:

$$\% \text{ TPH} = W2 / W1 \times 100$$

Where

$$\text{ppm} = \% \text{ TPH} \times 10000$$

W1 = Weight of hydrocarbon extracted in gram.

W2 = Sample weight in gram.

Figure 4-2 Auto soxhlet system

4.2.1.2 Produced water sample preparation

TPH was extracted from the PW samples using a liquid–liquid extraction technique. One litre of PW (V) sample was extracted by using 30 ml DCM in separator funnel. The extraction was repeated three times with 30 ml DCM. The extracts were dried by passing them through a drying column containing about 10 grams of sodium sulphate anhydrous then the solvent was completely evaporated (Venosa et al., 1996, EPA

method 3510C, 1996 and Li et al., 2005), the residue was dried and weighed (W) and TEH or DCM- extractable an organic material was then calculated as follows:-

$$ppm = \% \text{ TEH} \times 10000$$

$$\% \text{ TEH} = W / V \times 100$$

Where

W = Weight of hydrocarbon extracted in gram.

V = Sample volume in liter.

4.2.1.3 GC-FID condition

The extracted hydrocarbon from the soil and PW samples were subjected to gas chromatography to identify carbon distributions for the extracted hydrocarbon from the chromatogram and compare with carbon distributions of standard crude oil under similar GC conditions (EPA method 8015C 2000). The extracts were analyzed using gas chromatography type a Varian 3800 GC-FID system coupled to a Varian 8400 auto-sampler as shown in Figure 4-3, with a split/splitless injector used for the analysis of extracted hydrocarbon from the soil and PW. A fused silica column CP-5, 30 m x 0.32 mm ID with a film thickness of 0.25 mm was employed with helium as carrier gas. The column programme temperature was as follows: Initial temp: 40°C hold for 1 min, increasing to 300°C at 5°C min^{-1} and holding for 40 min (EPA method 8015C 2000). The chromatograms of the hydrocarbon extracted from the polluted samples were compared with chromatograms of fresh crude oil sample collected from the Nasser oilfield under the similar GC condition as the samples in order to identify the type of pollutants.

Figure 4-3 GC varian cp 3800

4.2.2 Determination of total petroleum hydrocarbon (TPH) in soil and produced water samples using infrared (IR) method

4.2.2.1 TPH measurement with TPH-IR

Spectroscopy plays an important role not only in the analysis of petroleum hydrocarbons but also for their characterization and identification. The spectroscopic technique utilizes the interaction between radiation and sample as a function of wavelength (λ). The spectroscopic technique measures the quantitative fraction of light that passes through a given sample. The concentration of a sample can be analyzed by comparing the absorbance of the sample at the given wavelength to the results of a series of standards. TPH is defined as anything extractable by a solvent, which is not removed by silica gel and can be detected by IR at a specified wavelength. Detection limits for the commonly used IR-based, EPA methods 413.2 and EPA 418.1 are approximately 1 mg/l in water and 10 mg/kg in soil. The US EPA methods 413.2 and 418.1 were used as the appropriate protocol to determine total recoverable petroleum hydrocarbons in the soil and PW. EPA methods 413.2 and 418.1 are the most common methods used for quantification of petroleum hydrocarbons in soil and water samples using infrared spectrometry (EPA method 8440 1996, Nadim et al., 2002 and ASTM D7066-04). After the extraction of oil and grease from a sample, silica gel is added to the extract. The method considers all 'oil and grease' materials that are not eliminated by silica gel adsorption as 'petroleum hydrocarbons'. Sodium sulphate is added to the sample during the extraction procedure and in the filtration process to eliminate any existing moisture in the sample. An Infrared spectrophotometer (TPH-IR) determines the extracted petroleum hydrocarbon by measuring the concentration of C-H bonds and their bonding frequency (EPA method 8440 1996, Nadim et al., 2002 and ASTM D 7066-04). The following spectroscopic technique was used to measure the concentrations of total petroleum hydrocarbon in soil and PW.

4.2.2.1.1 InfraCal TOG/TPH analyzer

The model CVH Infracal TOG/TPH Analyzer shown in Figure 4-4 is designed for use with EPA methods 413.2 and 418.1 that use Freon or polychlorotrifluoroethylene S-316 as solvents in the extraction procedure, where the concentration of a sample dissolved in an infrared transparent solvent is to be measured. A dual wavelenght detector is used in the TOG/TPH Analyzer to measure hydrocarbon concentrations at wavelength 3.4 micrometers (2940 cm^{-1}) with a reference at 2.5 micrometers (4000 cm^{-1}). The instrument was allowed to warm up for 1 hour prior to use and the analyzer calibrated with sets of mixed oil and solvent volumetrically.

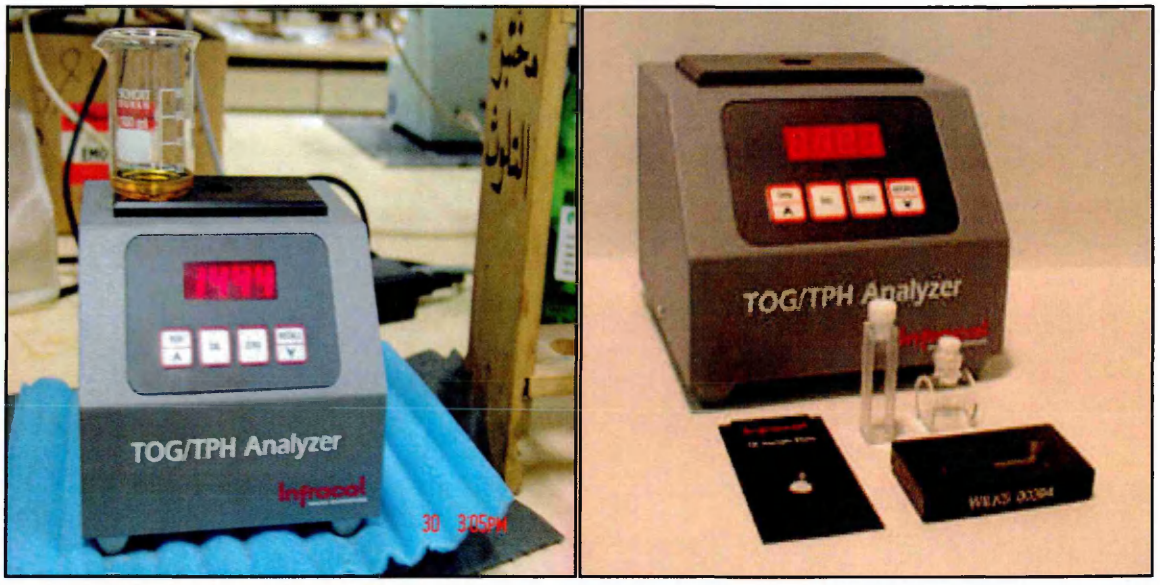
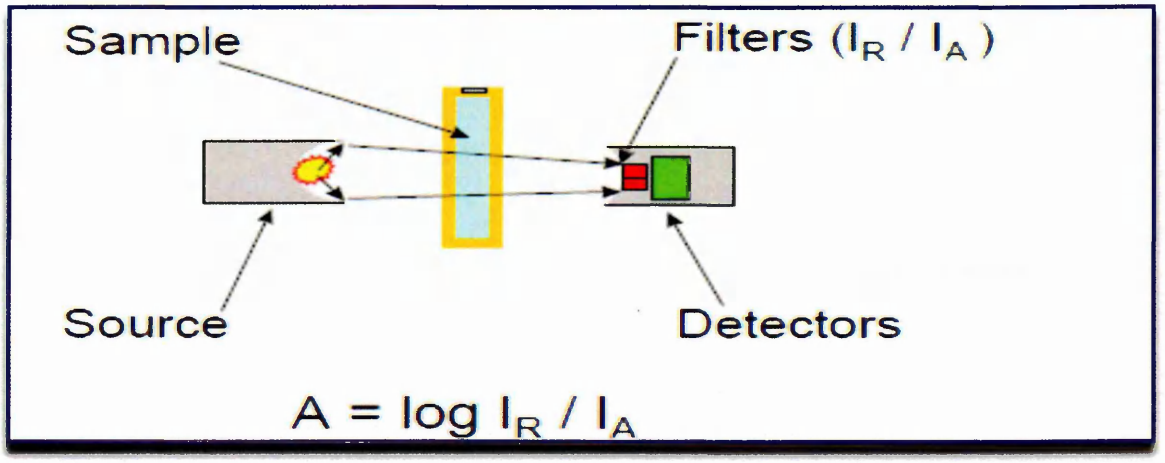


Figure 4-4 InfraCal TOG/TPH analyzer



Source: www.wilksir.com/pdf/ASTM-Method.pdf.

Figure 4-5 Measurements of IR absorption of oil with fixed filter analyzer

Standard B-heavy oil (specific gravity 0.895 at 20°C) known as the calibration oil obtained from Horiba, Ltd, Japan, was used for preparing standard solutions according to EPA-Approval protocol (EPA methods 413.2 and 418.1) at different concentrations (0 to 1000 mg/l) with polychlorotrifloroethylene (S-316) as solvent. The concentration of each standard was then measured by using the InfraCal TOG/TPH analyzer and the absorbance displayed on the screen recorded and a calibration curve created. Standards can be mixed either volumetrically or gravimetrically. Gravimetric determination is more accurate, yet requires a volumetric flask and scale accurate to 0.1 mg. The concentrations of the hydrocarbon extracted from soil and PW samples were then measured by using the InfraCal TOG/TPH analyzer and the concentration of TPH was directed displayed on screen as ppm. If the sample reading is outside of the standard curve, then the sample is diluted with S-316 and measured again until the concentration

falls within the standard range. According to TOG/TPH, CVH type analyzer manual, useful range for water test is 2-1000 ppm, while for soil the useful range is 3-5000.

4.2.2.1.2 Soil and produced water sample preparation

Polluted soil samples were directly transferred in weighted bottles and 5 gram of sample was taken and 1:10 up to 1:30 ratio of the polychlorotrifluoroethylene (S-316) solvent to the sample were used in the extraction. For PW samples 100 ml was transferred to glass sample bottles and the pH of the water was adjusted to less than two using HCl, than the water sample was transferred two separatory funnel. A 10 ml of solvent (S-316) was added. The bottles and the separatory funnel containing the sample and the solvent were capped and shaken vigorously for at least 5 minutes. The bottles and the separatory funnel containing the sample and the solvent were allowed to separate then the extracts which was in the bottom of the bottles and the separatory funnel were drained. The extracts were passed through silica gel to remove the polar organics and then 3 ml of the extract was transferred into the quartz cuvette and placed into the holder with the frosted side facing front to back; make sure that the cuvette is pushed down all the way to the stop. A beam of infrared light passed through the cuvette filled with extract and with either a filter set for C-H absorbance in a fixed filter infrared analyzer as shown in Figure 4-5. The extract absorbs infrared energy at 2940 cm^{-1} and then the amount of energy absorbed is proportional to the concentration of the hydrocarbon in the solvent. The infrared absorbance value was correlated directly to mg/l (i.e. for water) or mg/kg (i.e. for soil) and the concentration was displayed on screen. Hydrocarbon concentrations were determined by calculating the logarithm of the ratio of light transmission at reference wavelength to light transmission at analytical wavelength (EPA-Approval protocol EPA methods 413.2, EPA 418.1 and standard methods for the examination of water and wastewater 1985).

4.2.3 Total petroleum hydrocarbon (TPH) measurements in soil by using gas chromatography (GC-FID) method

Gas Chromatography (GC) is a chemical analysis instrument for separating chemicals in a complex sample. It is a commonly used analytical technique in industrial laboratories, environmental study and research. Since the 1940s it has been widely used in analytical and environmental studies since it is sensitive, fast and has a lot of stationary phases (Santos et al., 2002). GC is a separation method that relies on differences in partitioning behaviour between a flowing mobile phase and a stationary phase to separate the components in a mixture (McNair et al., 2011). Chromatographic procedures are referred to as displacement techniques which involve passing a mixture dissolved in a

mobile phase through an inert stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated. The two major mechanisms at work during a chromatographic separation are displacement and partition. In GC the mobile phase is always a gas (mostly N_2, H_2 or He). The stationary phase in gas solid chromatography or adsorption chromatography is a porous polymer solid, while in gas liquid chromatography or partition chromatography the stationary phase is a mostly viscous gum like liquid. Packed columns are completely filled with a packing, liquid stationary phases being coated onto an inert support. A column holds the stationary phase and the mobile phase carries the sample through it. Sample components that partition strongly into the stationary phase spend a greater amount of time in the column and are separated from components that stay mostly in the mobile phase and pass through the column faster, when the components elute from the column they can be quantified by a detector (McNair et al., 2011). The TPH analysis typically uses infrared spectroscopy or GC with a flame ionization detector (FID) or a photoionization detector (PID) to provide a measurement of the bulk concentration of PHs present in a sample (Nadim et al., 2002). GC based methods are currently the preferred laboratory methods for TPH measurement because they detect a broad range of hydrocarbons, they provide both sensitivity and selectivity, and they can be used for TPH identification as well as quantification (Weisman 1998). TPH measurement by GC methods is defined as everything extractable by a solvent and detectable by gas GC/FID within a specified carbon range. The advantage of GC methods is that they provide information about the type of petroleum in the sample in addition to measuring the concentration. Detection limits can be as low as 0.5 mg/l in water or 10 mg/kg in soil (Weisman 1998).

4.2.3.1 Sample preparation

About 10 to 12 g of contaminated soil were weighed (W1) and mixed with 10 grams of sodium sulphate anhydrous as a drying agent to reduce some of the moisture present in the soil and placed in an extraction thimble (EPA method 3550C 2007). 250 ml aliquots of DCM were added to the round flask of the soxhlet unit, along with a few anti-bumping granules. The thimble was inserted into the soxhlet head, and the unit fitted together. Samples were refluxed for 24 to 48 hours on heating mantles, and then contents were allowed to cool, DCM was allowed to drain into the soxhlet head and then the thimble was removed and discarded, the extract was then poured into a beaker, and the flask washed out with DCM. The DCM was then left to evaporate down to dryness at room temperature, then the residue was dried and weighed (W2) and total

extractable hydrocarbon (TEH) or DCM extractable organic materials was then calculated (EPA Method 9071B 1998) as follows:

$$\% \text{ TEH} = W2 / W1 \times 100$$

$$\text{ppm} = \% \text{ TEH} \times 10000$$

Where

W1 = Weight of soil sample.

W2 = Weight of hydrocarbon extracted in gram.

4.2.3.2 Sample cleanup

Sample extracts were processed through a cleanup and fractionation procedure designed to separated the extract into two fractions. The first fraction, total saturated petroleum hydrocarbons (TSPH) consisted of saturated hydrocarbons such as n-alkanes, branched alkanes and cycloalkanes. The second fraction, total aromatic petroleum hydrocarbons consisted of aromatic hydrocarbons which include the PAHs (Mills et al., 1999). A standard open liquid chromatography column was prepared (A 20-30 cm glass column length and 10 to 15 mm bore) with glass wool (2-3 cm), 15 gram of activated silica gel (70- 230 mesh) was added with 6 gram of sodium sulphate into the top of the column as a drying agent to reduce some of the moisture present in the soil. The column was then eluted with a minimum of 25 ml of hexane to prime the column to ensure the column bed was wet, and that it had not dried out after elution (EPA method 1664 1999, EPA method 3630B 1996 and Mills et al., 1999). The sample extract redissolved in hexane (2 ml) was then poured onto the top of the column, using a small filter funnel. The column was eluted with a further 25 ml of hexane to collect the TSPH, and then the hexane was evaporated to dryness and the residue was dried and weighed (W3). The TSPH were calculated gravimetrically as follows:

$$\% \text{ TSPH} = W3 / W1 \times 100$$

$$\text{ppm} = \% \text{ TSPH} \times 10000$$

Where

W1 = Weight of soil sample.

W3 = Weight of extracted hydrocarbon in gram.

Then the residue was dissolved in hexane again and the volume was made up to a 25 ml volumetric flask, the sample was then ready for TPH analysis using gas chromatography (EPA method 8015 C 2000).

4.2.3.3 Instrumentation

The hydrocarbon extracts from soil were injected into a Perkin Elmer Clarus Model 500 gas chromatograph equipped with Perkin Elmer auto-sampler and flame ionization detector with a split/splitless injector (90:1) and nitrogen as carrier gas, for the measurement of TPH in soil samples. The capillary column used was TM-5 (15 m x 0.32 mm i.d. x 0.25 μm film thickness). The injector and detector temperatures were set at 280°C and 330°C, respectively. Using temperature programming, the compounds could be eluted from the column separately. The temperature program was the following: the initial temperature was set to 80°C and held for 3 min. The temperature was increased 20°C /min to a final temperature of 320°C. The final temperature was held for 8 min, bringing the total analysis time to 23 min (EPA Method 8015C 2000 and Peramaki et al., 2010).

4.2.3.4 Calibration of the gas chromatography (GC)

For initial calibration of the GC, the analytical standard solution of total petroleum hydrocarbon (mixed TPH) mixture dissolved in hexane with concentration of 2000 μg/ml obtained from Supleco were prepared at different concentrations (i.e 10, 20, 50, 75 and 100 ppm). The concentrations used of TPH standard was used to fit into the working range of the detector and to cover the expected range of concentrations found in real samples. Figure 4-6 shows the calibration curve known TPH standard concentration against the total peak area of the each standard in order to identify the concentrations of total petroleum hydrocarbon in unknown sample. The standard solution and the extracted hydrocarbon from the samples were injected into the GC under the similar condition. For TPH quantitation, in sample contains high petroleum hydrocarbon, a standard stock solution (70000 mg/l) was prepared by mixing equal amounts (by mass) of fresh crude oil (from the Nasser oilfield, Libya) in dichloromethane (HPLC grade). Selected volumes of this solution were further diluted with dichloromethane to give a series of working standards with TPH concentrations of 10000, 20000, 30000, 40000, 50000, 60000 and 70000 mg/l as shown in Figure 4-6. The concentrations of TPH standard used were within the working range of the detector and covered the expected range of concentrations found in the real samples. The concentrations of hydrocarbons can be calculated in the sample using the equation:-

$$\text{TPH concentration (in ppm)} = \frac{\mathbf{As \times Vt \times DF}}{\mathbf{CF \times Vi \times We}}$$

Where

As = Sample peak area

Vt = Total volume of extract

DF = Dilution Factor

Vi = Volume of extract injected

We = Weight of soil extracted

$$CF = \text{Calibration Factor} = \frac{\mathbf{Ast}}{\mathbf{Cst}}$$

Ast= Standard peak area

Cst = Standard concentration

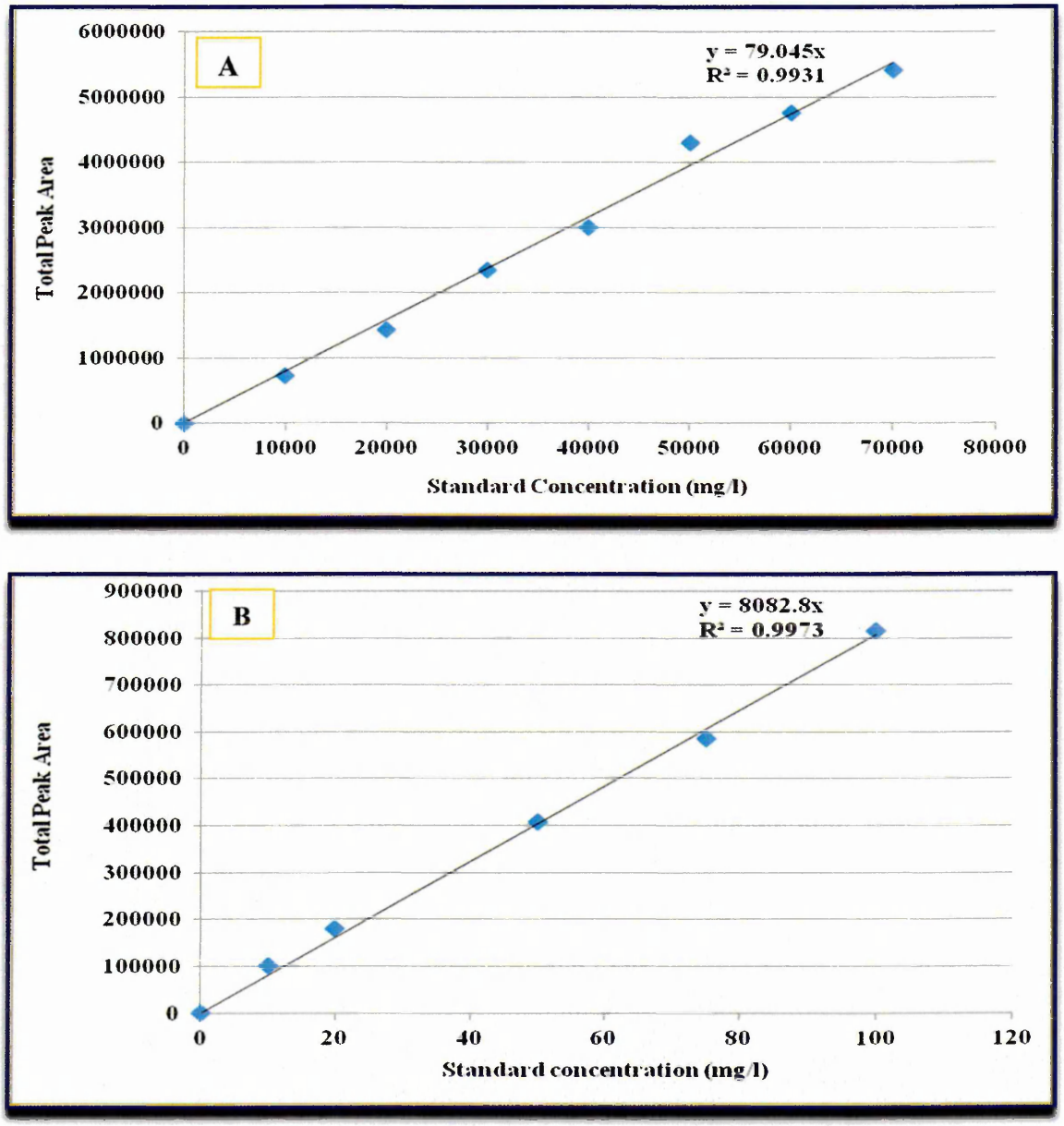


Figure 4-6 Total petroleum hydrocarbons standard calibration curves A) Nasser oilfield crude oil B) Mixed TPH standards

4.2.4 Determination of BTEX in produced water using SPME and GC-FID

As described previously the extraction of crude oil and natural gas in the petroleum industry is usually associated with large quantities of PW. PW is a complex mixture consisting of inorganic and organic compounds of natural origin. However, the most

harmful contaminants present in PW are BTEX compounds and polycyclic aromatic PAHs. The most abundant hydrocarbons in PW are the one ring aromatic hydrocarbons, known as BTEX (Neff et al., 2002, Deriszadeh et. al., 2010 and Sundt et al., 2011). BTEX are the volatile components commonly associated with petroleum products and general contaminants in ground water. In fact, these chemicals, because of their volatility and aqueous solubility, are widely distributed in the environment and can cause a hazard to public health. BTEX entering our environmental system cause serious environmental problems since they all have acute and long term toxic effects. In addition to the acute toxicity, benzene is known to be carcinogenic (Maiolini et al., 2010, Hosseinzadeh et al., 2011, and Bianchin et al., 2012). BTEX is volatilized from the surface of the soil after a spill, and these monoaromatic hydrocarbons are thought to be primarily a human health concern. Risks to groundwater are considered the next most important due to the high relative mobility of BTEX through soils column (Gaujac et al., 2008, Maiolini et al., 2010, Hosseinzadeh et al., 2011, and Bianchin et al., 2012).

Many analytical methods are available with low detection limits to measure the BTEX in water and soil. BTEX were analyzed by direct aqueous injection and gas chromatography with flame ionization detector (GC–FID). BTEX in liquid samples have also been analyzed by solid phase microextraction [SPME] and GC–FID and at present, gas chromatography–mass spectrometry (GC–MS) is widely used to determine BTEX in different water samples (Maiolini et al., 2010, Farajzadeh et al., 2008, and Bianchin et al., 2012). In many cases, compounds in environmental samples are present in low concentration levels and commonly used detectors in GC are not able to detect them. For this reason, sample preparation becomes essential. In water samples, a pre-treatment is often necessary to isolate the components of interest from sample matrices, to purify and concentrate the analytes. Liquid–liquid extraction, solid-phase extraction and purge-and-trap techniques were used for determination of BTEX in the past. Today special attention is paid to sample preparation methods in environmental analysis that ensure reduction of the quantity of solvents or even their complete elimination in the course of the analytical procedure. Further, the number of operations and processes involved in the sample preparation stage should be kept to a minimum. Solid phase microextraction is a very convenient technique used for the analysis of volatile and semivolatile compounds in liquid samples (Gaujac et al., 2008, Hosseinzadeh et al., 2011 and Laaks et al., 2012). However, SPME, as rapid, selective and solvent-free techniques, are more and widely used for analysis of trace BTEX in soil and water samples. In the late 1980s SPME was developed by Janusz Pawliszyn. This is a simple

technique for VOC that requires no solvent and allows both extraction and concentration to be achieved in a single step. The most common applications, for the use of SPME are in the field of measuring volatile organic compounds (VOCs) such as BTEX, semivolatile organic compounds such as PAHs and pesticides analysis (Gregg et al., 2006, Gaujac et al., 2008, Hosseinzadeh et al., 2011, and Laaks et al., 2012). SPME has been more and more widely used in sample preparation, especially since the first fibres became commercially available in 1993. The SPME technique is based on the establishment of an equilibrium between the analyte and a fused silica fibre coated with a stationary phase. This can be a liquid polymer, a solid sorbent, or a combination of both. Although a stir bar could accelerate the mass transfer of analytes through the aqueous matrix, the time of extraction, the temperature and headspace of sample vial have an effect on the partition equilibrium. The analyte is then desorbed from the fibre into a suitable separation and detection system, usually a gas chromatograph (Pawliszyn 1997, Dietz et al., 2006 and Laaks et al., 2012). This section will describe the measurement of BTEX in PW using SPME and GC-FID.

4.2.4.1 Apparatus

A manual SPME holder and 25 ml sample vial, a 100 μm polydimethylsiloxane (PDMS) fibre assembly, gas chromatography flame ionisation detector (GC-FID) and mixture BTEX standard in methanol at 2000 μg/ml obtained from Supelco, UK, were used in the measurements of BTEX in produced water.

4.2.4.2 Standard solutions preparation and microextraction procedure

Stock solutions of BTEX (2000 μg/ ml) were prepared by dissolving a calculated amount of each BTEX compound in methanol to obtain working standards. A series of BTEX standard solution (50, 100, 200, 300, 400 and 1000 ppb) were prepared by spiking the appropriate amount (i.e. 12.5, 25, 50, 75, 100 and 250 μl of 100 ppm BTEX in HPLC grade methanol respectively) to the 25 ml organic free water in the sample vial which give spiked water solutions. In order to avoid evaporative loss of the BTEX compounds, all solutions were prepared and used prior to the analysis (CH424 instrumental analysis lab 1998 and Li et al., 2010).

In order to obtain standard BTEX calibration curves the series of BTEX spiked water samples were treated with SPME and analysis by GC-FID. For extraction, 25 ml from the lower BTEX standard in water (50 ppb) was taken and transferred to sample vial containing a magnetic stirrer. The vial containing the sample was sealed and then, a syringe needle containing the 100 μm PDMS fibre penetrated the vial stopper. The SPME fibre was inserted into headspace of the vigorously stirred sample at 40°C and

left for 15 min at 1000 rpm. When the adsorption step was complete, the fibre was withdrawn into the needle, and the syringe was removed from the vial. The final step was thermal desorption of the analytes from the solid-phase microextraction fibre in the GC injector at 270°C for 2.5 min to adsorb the analytes (CH424 instrumental analysis lab 1998, Farajzadeh et al., 2008, Gaujac et al., 2008 and Li et al., 2010). The other concentrations of BTEX standards prepared were then carried out under the same experimental condition in order to construct the calibration curves by plotting the peak area against the concentration of BTEX used (Figure 4-7). According to the supplier's instructions the SPME was conditioned by desorption for 5 minutes in a GC injector in order to flush out any of the fibre impurities before use. The calibration curves for each of the BTEX compounds were obtained by plotting the peak area vs. concentration of each standard used as shown in Figure 4-7.

4.2.4.3 Procedure for BTEX extraction from produced water using SPME

The extraction was performed using 25 ml sample vial containing a magnetic stirring bar. 25 ml aliquots of the unfiltered PW samples were placed into the sample vials, which were then sealed using butyl rubber stoppers wrapped with Teflon sealing tape. A syringe needle containing the 100 μm PDMS fibre penetrated the vial stopper, and the fibre was then lowered into the headspace located above the sample solution by depressing the plunger. The solution was vigorously stirred at 40°C for 15 min at 1000 rpm. When the adsorption step was complete, the fibre was withdrawn into the needle, and the syringe was removed from the vial. The final step was thermal desorption of the analytes from the SPME fibre into the GC injector at 270°C for 2.5 min. The SPME was conditioned by desorption for 5 minutes in a GC injector in order to flush out any of the fibre impurities before the next use (Gaujac et al., 2008 and Li et al., 2010).

4.2.4.4 GC-FID conditions

A Varian 3800 gas chromatograph equipped with a flame ionisation detector and a splitless injector was used for the quantification of BTEX. A chrompack capillary column [CP. Sil-8] CP with 60 meter length × 0.32 mm internal diameter and 0.40 μm film thickness supplied by J and W Scientific (UK) was employed with helium as carrier gas at flow rate of 1.5 ml/min and 1μl injection volume. The column temperatures programs of the GC system were as the follows: initial temperature 35°C for 1 min, and then to 300°C at a rate of 5°C /min and then held for 4 min to give a total run of 38 min. The injection and detector were operated at 270 and 300°C respectively (Gaujac et al., 2008 and Li et al., 2010).

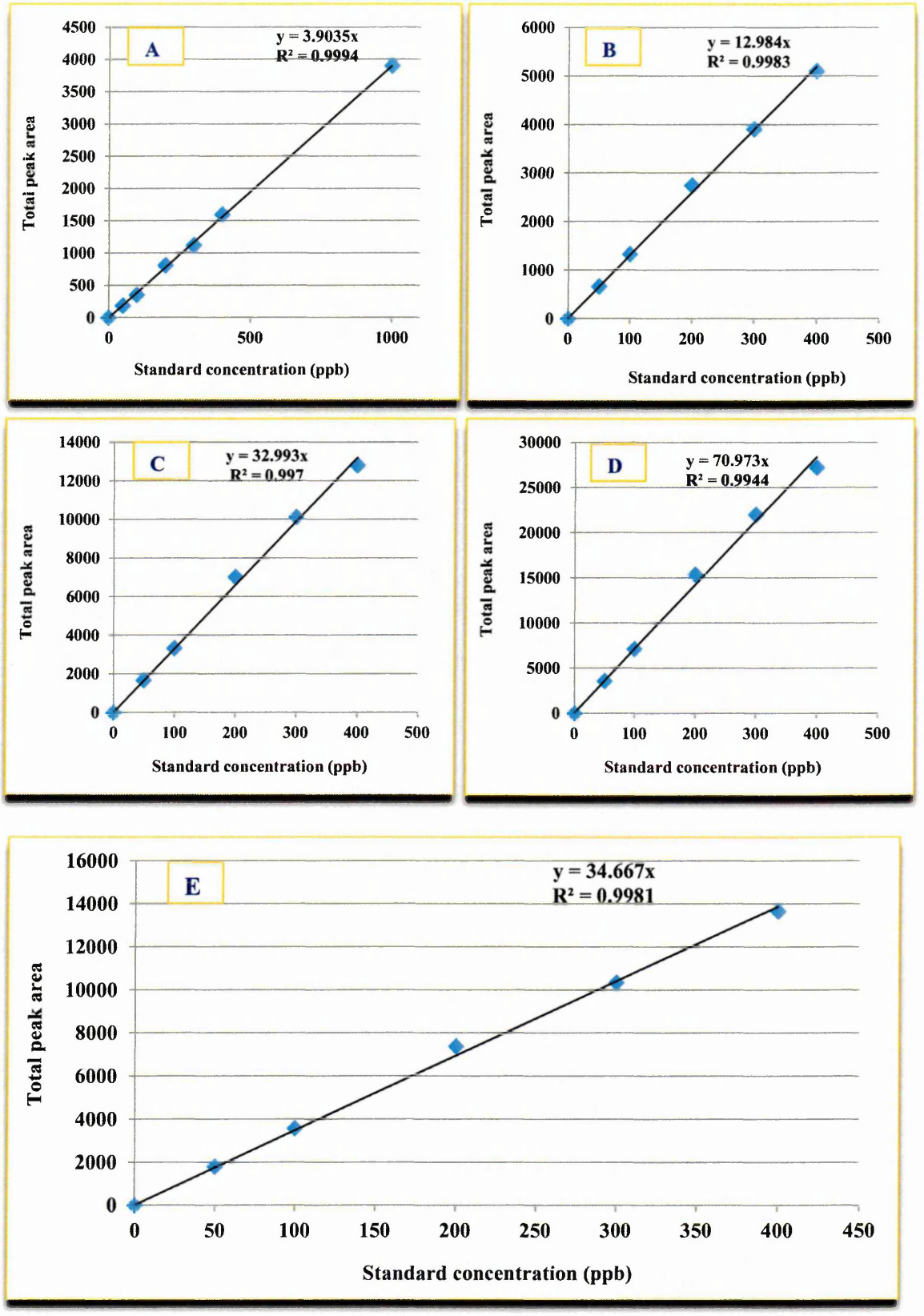


Figure 4-7 A calibration curve plot showing linearity of each BTEX standard A) Benzene, B) Toluene, C) Eethylbenzene, D) P+M -Xylene and E) O-Xylene

4.2.5 Determination of polynuclear aromatic hydrocarbons (PAHs) in soil and produced water by using gas chromatography mass spectrometry (GC-MS)

PW is a complex mixture consisting of inorganic and organic compounds of natural origin. The most harmful contaminants present in PW are BTEX and PAHs. PAHs are composed of two or more fused benzene rings also called polynuclear aromatic hydrocarbons, are the petroleum hydrocarbons of greatest environmental concern in PW, because of their toxicity (Neff et al., 2002 and Sundt et al., 2012).

Polynuclear aromatic hydrocarbons (PAHs) are an important group of organic pollutants present in water, soil and air. The group contains of several hundred compounds which have two or more aromatic rings in their structure. As a results of their widespread distribution in the environment, and due to the toxic, carcinogenic and mutagenic nature of certain PAHs their concentration is of a rising environmental concern (Anyakora et al., 2006, Yang et al., 2011 and Bianchin et al., 2012). Sixteen priority PAHs were defined by the USEPA as priority environmental pollutants. Some PAHs are considered to be more harmful than the others; since there is greater possibility of people being exposed to them, and hence their distributions in the environment and the possible exposure of humans are the focus of much attention. Many high molecular mass PAHs are also suggested to be probable human carcinogens. Both natural and industrial sources contribute PAHs to the environment (Anyakora et al., 2006, Yang et al., 2011 and Srujana et al., 2012).

PW contains measurable concentrations of 2- to 5- ring polynuclear aromatic hydrocarbons (PAHs) which are discharged into the open environment (Orszulik 2007). PAHs may be transformed to even more toxic compounds by chemical reactions such as nitration, sulfonation or photooxidation. PAHs have low volatility and low water solubility (solubility in tap water of about 0.001 mg/ l). When found in water samples they are generally adsorbed onto dissolved solid, which can be attached to them (Valavanidis et al., 2008 and Yang et al., 2011).

The determination of the concentration of PAHs at contaminated sites is of critical concern because of their toxicity to humans and their effects on soil organisms and plants. Many analytical techniques have been developed and applied for the monitoring of these compounds in the natural environment including immunoassay tests, although PAHs are mainly determined by chromatographic methods. Liquid chromatography (LC) with fluorescence detection, GC with FID has been widely applied. Due to the lack of selectivity of the FID, it is being replaced by mass spectrometry (MS) detection using several analyzers such as single, triple quadrupoles or ion trap, providing good

selectivity, low limits of detection (LODs) and reliable confirmation of PAHs. High performance liquid chromatography coupled with ultraviolet-diode array detection (HPLC-UV-DAD) or fluorescence detection (FLD) and GC-MS are the most common analytical methods for determination of PAHs in environmental samples. However, sample preparation and especially extraction is an important step in PAHs analysis, because these hydrophobic compounds are strongly sorbed to the soil material (Bonilla et al., 2009, Yang et al., 2011 and Bianchin et al., 2012). The following analytical equipments and standard test methods are used for the measurement of PAHs in soil and PW samples.

4.2.5.1 Material and method

4.2.5.1.1 Sample collection

Whole bulk PW samples were collected from the Nasser oilfield, Libya (namely Spt1, Spt2, Spt3 and Spt4). Four PW samples were taken from the Nasser oilfield disposal pit. The samples were collected from the locations which are close to the discharge point of the effluent as shown in Figure 2-17 and 2-18-. The samples were collected in 2 litre polypropylene bottles; the samples were acidified at the site by using HCl to avoid any biodegradation by microorganisms and transferred to the laboratory and stored at 4°C until use.

4.2.5.1.2 Preparation of PAHs standard solution

All solvents (DCM, n-Hexane, etc.) used for the analysis were of HPLC grade and were obtained from Sigma Aldrich. Silica gel (70–230 mesh) and sodium sulphate anhydrous were of analytical grade and were obtained from Sigma. Standard mixtures of 16 PAHs dissolved in DCM in concentrations of 2000 µg/ml (QTM PAH mix) including naphthalene (NAP), acenaphthene (ACL), acenaphthylene (AC), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FA), pyrene (PY), benz(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbFA), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3 cd)pyrene (IP), dibenz(a,h)anthracene (DBahA), and benzo(ghi)perylene (BghiP) were obtained from Supelco UK. Five standard solutions each containing 16 target compounds were prepared by diluting the mixed standard (QTM PAH mix) to required concentrations with HPLC grade DCM.

Standard solutions of PAHs were obtained by appropriate dilution of PAH in DCM. The concentrations of the mixed PAH standard solutions used for determining the linearity were 2, 5, 10, 15 and 20 mg/l. These were prepared and transferred to a capped and sealed vial and stored at 4°C until analysis. Each standard was injected into GC to find

out the retention time and total peak area of each PAH. The calibration curves were prepared for each PAH for concentration ranges between 2 ppm to 20 ppm. An example of a calibration curve is presented in Figure 4-8 for Napthtalene (NAP) and Dibenz [a,h] anthracene (DBahA), where the total peak area is plotted against the standard concentration. The PAHs considered in this study are shown in Table 4-1. Liner calibration graphs were obtained for all sample concentration ranges that were determined in soil and PW. These calibration datas were used to determine the cocenration of each PAH found in the samples.

Table 4-1 PAHs studied, showing their formula and structure

Compound	MF	Quantification ion (m/e)	Compound	MF	Quantification ion (m/e
Naphthalene (NAP)	$C_{10}H_8$	128	Benz[a]anthracene (BaA)	$C_{18}H_{12}$	228
Acenaphthylene (ACL)	$C_{12}H_8$	152	Chrysene (CHR)	$C_{18}H_{12}$	228
Acenaphthene (AC)	$C_{12}H_{10}$	154	Benzo[b]fluoranthene (BbFA)	$C_{20}H_{12}$	252
Fluorene (FL)	$C_{13}H_{10}$	166	Benzo[a]pyrene (BaP)	$C_{20}H_{12}$	252
Phenanthrene (PHE)	$C_{14}H_{10}$	178	benzo(k)fluoranthene (BkF)	$C_{20}H_{12}$	252
Anthracene (AN)	$C_{14}H_{10}$	178	Indeno[1,2,3-cd]pyrene (IP)	$C_{22}H_{12}$	276
Fluoranthene (FA)	$C_{16}H_{10}$	202	Dibenz[ah]anthracene (DBahA)	$C_{22}H_{14}$	278
Pyrene (PY)	$C_{16}H_{10}$	202	Benzo[ghi]perylene (BghiP)	$C_{22}H_{12}$	276

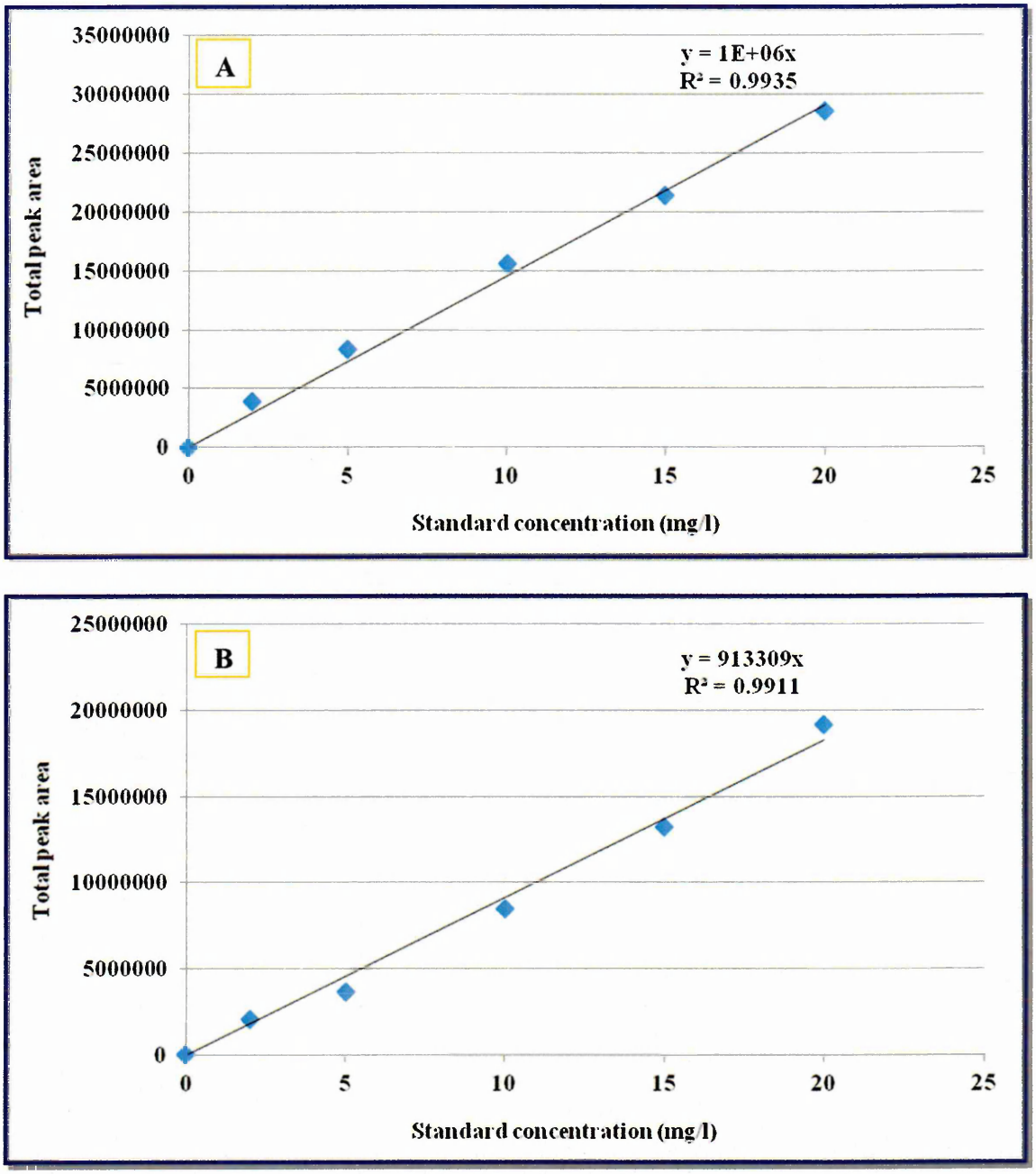


Figure 4-8 A calibration curve plot showing linearity of PAHs standard solution A) Naphthalene, B) Dibenz (a, h) anthracene

4.2.5.1.3 Extraction of polycyclic aromatic hydrocarbons (PAHs) from PW

An analytical aliquot of 1000 ml of PW was transferred to separating funnel and 30 ml of dichloromethane were added to the mixture. It was shaken for a few minutes and allowed to separate and settle. The DCM extract was collected into a 250 ml Erlenmeyer flask and the process was repeated by adding a second 30 ml of DCM to the sample in the separating funnel. The extraction was performed for a third time with another 30 ml of DCM in the same separating funnel and the extracts were combined in the Erlenmeyer flask. Then the DCM was evaporated under nitrogen (EPA method 625,

1996, and EPA method 3510C, 1996). The residue was re-dissolved in hexane and transferred to a chromatographic glass column (20-30 cm long x 2 cm internal diameter) packed with glass wool (2 cm) and 15 gram of silica gel (70-230 mesh, activated for 16 hr. at 130°C) with 5 gram sodium sulphate anhydrous on the top of the column. The column was then eluted with 25 ml of n-hexane to prime the column to ensure the column bed was wet. The sample was then poured onto the top of the column and 25 ml of n-hexane was added to the column to remove the aliphatic fraction. All eluent from the hexane application was discarded. PAHs were then desorbed with 25 ml of DCM, and the extract was collected in the flask and the solvent was reduced to approximately 2 ml by nitrogen blow down at ambient temperature in a fume hood. The extract was transferred to GC vials (EPA method 3630C 1996 and Dorea et al., 2007). PAHs concentration was measured by using GC-MS. The PAHs identification and quantification based on ion fragmentation and retention time compared to that of the PAHs standard used (EPA method 8270D 1998 and Dorea et al., 2007).

4.2.5.1.4 Extraction of polycyclic aromatic hydrocarbons (PAHs) from soil

Soil samples were freeze dried and homogenized by hand mixing. The extraction of PAHs of interest was made by taking ten grams of mixed and homogenized soil sample and extracted with 20 ml DCM in an ultrasonic water bath for 20 min followed with mechanical homogenization for 10 min at room temperature. Then the soil samples were extracted again by adding a second 20 ml of DCM for 15 min. The extraction was performed for a third time with another 20 ml of DCM, and then the extracts were combined and filtered in a suitable flask. The collected solution was passed through a glass column contain 10 grams of sodium sulphate anhydrous. The final extract was concentrated to 0.5 ml using a rotary evaporator and evaporated to dryness under a gentle stream of nitrogen. (EPA method 3550C 2007 and Dabrowska et al., 2003).

4.2.5.1.5 SPE for clean-up of extraction solution

The residue was re-dissolved in hexane (15 ml) and loaded onto the top of the discovery DSC-18, solid phase extraction cartridges (500mg, 3 ml), and previously preconditioned with 4 ml ultrapure water, 3 ml methanol followed by 3 ml hexane. The extracted sample (15 ml) was applied to the top of the tube and drawn through the packing bed at 1 ml/min, and then the column was washed with 3 ml ultrapure water. Finally the cartridge was allowed to dry then the PAH was eluted with 5 ml DCM. The collected eluent in DCM was evaporated down to 1 ml under a gentle stream of nitrogen and the PAHs concentration was measured by using GC–MS in full scan mode and in selected ion mode (EPA method 3630C 1996, EPA method 8270D 1998 and Wang et al., 2004).

4.2.5.1.6 Gas chromatography- mass spectrometry (GC-MS)

The sample extracts were analyzed for the target PAH compounds by GC/MS using a Hewlett-Packard 5890 series II, coupled to a 5971A mass selective detector with a Hewlett-Packard 6890 autosampler (Hewlett-Packard CA USA). Separation was performed on an HP5-MS fused silica capillary column with 30 meter length × 0.25 mm internal diameter and 0.25μm film thickness. Helium was used as carrier gas at a flow rate of 1 ml/min. Injection volume was 1μl in the split/splitless mode. The temperatures programme of the GC system was as the following: injection temperature was 280°C, initial temperature at 50°C for 4 min, ramped to 260°C at a rate of 10°C /min, and held for 2 min, and then the temperature was increased to 300°C at a rate of 6°C /min held for 5 min to give a total run of 38.67 min. The MS detector was operated in the selected ion mode (SIM). The PAHs identification and quantification was based on ion fragmentation and retention time compared to that of the PAH standard used (EPA method 8270D 1998 and Wang et al., 2004). To obtain standard calibration curves different concentrations of PAHs were analysed by GC-MS under identical conditions to the sample and then the calibration curves were plotted using known PAHs concentration against the peak area of each individual compound as shown in Figure 4-8. The concentration of each compound was determined by comparing the peak area obtained in the samples with these data.

4.3 Results and discussions

This chapter describes results obtained from a survey of PW from the Nasser oilfield for the levels of TPH, BTEX and PAHs in the production facilities and disposal pit. This oilfield was selected because it contributes a high percentage of the total amount of PW discharged into surface open environment. Furthermore the results of all standards calibration curves for TPH, BTEX and PAHs are also presented.

4.3.1 Total petroleum hydrocarbons (TPH) analysis

TPH is an estimation of the amount of hydrocarbons present in a sample and is calculated using n-alkanes ranging from C_8 to C_{40}. This extends from the volatile hydrocarbons compounds with the lower boiling to the non-volatile compounds found at the higher boiling point range (Gawad et al., 2010).

A number of analytical techniques, based primarily on gas chromatography (GC), gas chromatography / mass spectrometry (GC–MS), infrared spectroscopy (IR) and liquid chromatography (LC) have been developed and employed by researchers and regulatory bodies to identify, characterize and measure petroleum contamination in soils, sediments and water (Mills et al., 1999 and Villalobos et al., 2008). This part describes a study in which three analytical techniques including IR, GC/FID, and gravimetric methods were used to identify and characterize the TPH in soil and PW samples to give a comparison on their concentrations.

The TPHs were measured in soil and PW using different analytical techniques (TPH-IR, TPH-GC and TSPH) and Dichloromethane total extractable hydrocarbons (TEH) and the results as follow:

4.3.2 Determination of hydrocarbons (TPH and TEH) in produced water

Hydrocarbons are the other constituents carried by PW which are presented in different forms, free oil phase (Oil sheens), dispersed oil and dissolved forms but the measurement was carried out only for the dissolved hydrocarbons. Six PW samples were subjected to the total petroleum hydrocarbon (TPH) and total extractable hydrocarbons (TEH) analysis. Tables 4-2 and 4-3 show the concentrations of TPH and TEH in PW. These were determined throughout the production process and at different points in the disposal pits. The average data obtained for triplicate samples for the TPH concentrations of the PW is shown in Table 4-2. The average values of TPH recorded for sampling points which have been taken from settled tanks outlet (TA) are higher (2.9±0.20 mg/l) than other sampling point (Pump, Spt1 to Spt4), which ranged, according to the point location, from 1.2±0.10 to 2.9±0.20 mg/l as shown in Figure 4-9. The concentrations of TPH in these huge amounts of PW can have a toxic impact on the

ecosystem during long term PW disposed. However, the levels recorded in this study for the total TPH in PW are much lower than those found in some other locations, such as in the PW from Campos Basin, Brazilian Petroleum Company, Rio de Janeiro State, Brazil, the total TPH by using GC method is 49 mg/l (Campos et al., 2002).

Gawad et al., 2010 has reported the concentrations of TPH in produced water collected from Marmul oilfield Main Production Station, Sultanate of Oman, is in the range of 50 to 250 mg/l with an average of 100 mg/l used IR method and Tellez et al., 2002 who found the concentrations of total petroleum hydrocarbon in PW taken from an oil separation facility located in south western US within the mineral rich Permian Basin Lea County, New Mexico in the range of 126 mg/l ±30 used IR method.

Multon et al., 2006 evaluated the concentration of total petroleum hydrocarbons for produced water samples obtained from two independently operated heavy oil production sites in western Saskatchewan, Canada and had found the concentration in the range of 212-264 mg/l. In addition, Lu et al., 2009 has reported the concentrations of TPH in PW obtained from the treatment system for the south water injection station of the Qinghe Oil Production Plant, Shengli Oilfield, China, in the range of 14 to 32 mg/l with an average of 23 mg/l used GC method

Table 4-2 Concentrations of TPH in produced water samples

Sample Code	Concentration in mg/l			
	Minimum Min.	Maximum Max.	Average Avg.	Standard deviation STDEV
TA	2.7	3.1	2.9	± 0.20
Pump	1.6	2.3	1.9	± 0.36
Spt1	1.1	1.4	1.3	± 0.14
Spt2	1.8	2.1	1.9	± 0.17
Spt3	1.0	1.4	1.2	± 0.20
Spt4	1.2	1.4	1.3	± 0.10

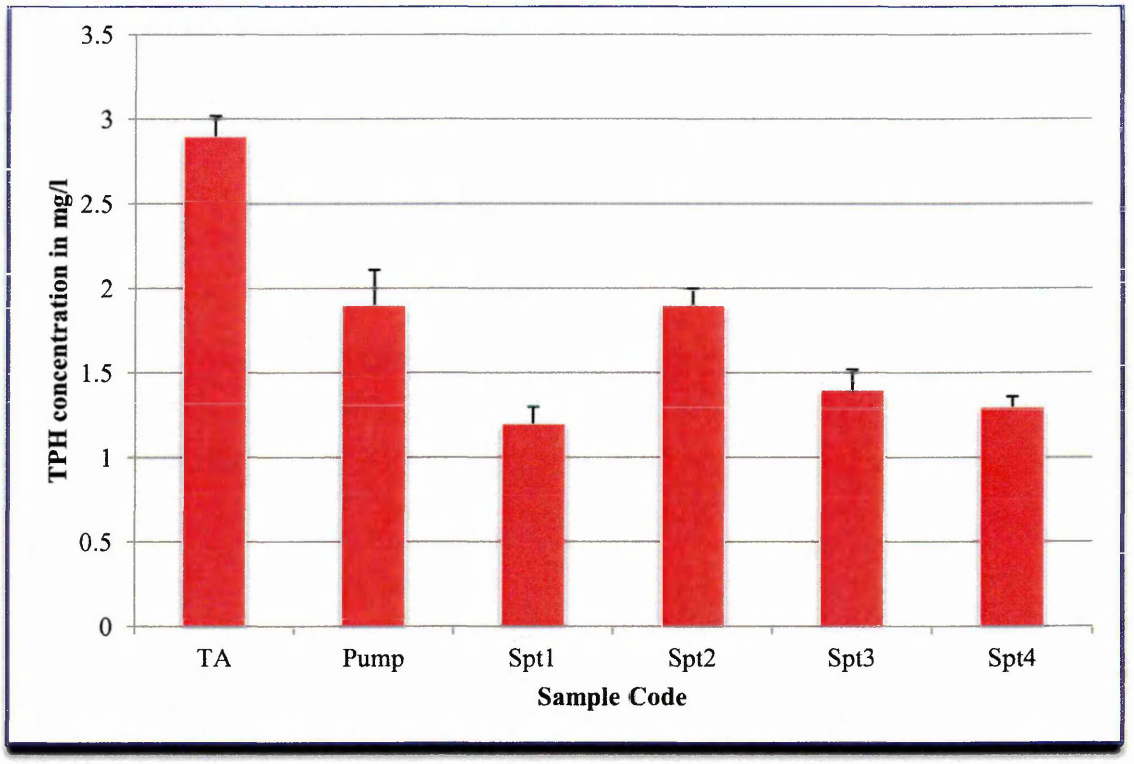


Figure 4-9 Average total petroleum hydrocarbons (TPH) concentration in produced water samples

Total extractable hydrocarbons (TEH) represents total materials that are extracted with DCM as solvent and can be defined as the sum of aliphatic, aromatics and other organic found in PW (Stromgren 1995). The average data obtained for triplicate samples for the TEH concentrations of the PW is shown in Table 4-3. The TEH values in PW vary during the process until the final disposing pit. The average concentrations of TEH in PW ranged from 3.4 mg/l to 13.5 mg/l as shown in Table 4-3. Concentrations of TEH in the settled tank (TA) ranged from 12.4 to 14.1 mg/l with an average of 13.5 mg/l ± 0.92. The pump station contains TEH concentrations ranged from 10.5 to 12.9 mg/l with an average of 11.7 mg/l ± 1.20. The concentrations of TEH in the PW samples taken from disposal pit namely, Spt1, Spt2 Spt3 and Spt4 start to contains TEH in the range of 3.0 to 3.8 mg/l with an average of 3.4 mg/l ± 0.40 for Spt1, 4.8 to 5.3 mg/l with an average of 5.1 mg/l ± 0.26 for Spt2, 3.8 to 4.5 mg/l with an average of 4.2 mg/l ± 0.36 for Spt3 and 3.6 to 4.1 mg/l with an average of 3.8 mg/l ± 0.26 for Spt2 as shown in Table 4-3. High average concentrations of TEH were observed in the location of settled tanks outlet (TA), where the PW is in the earlier steps of productions, before discharging to the pits, while lower TEH were found at sampling location Spt1 (i.e. pit sample). A ranking of the sampling point at the Nasser oilfield according to the TEH are TA > Pump > Spt2 > Spt3 > Spt4 > Spt1 as shown in Figure 4-10.

Table 4-3 Concentration of total extractable hydrocarbons (TEH) in PW samples

Sample Code	Concentration in mg/l			
	Minimum Min.	Maximum Max.	Average Avg.	Standard deviation STDEV
TA	14.1	12.4	13.5	± 0.92
Pump	12.9	10.5	11.7	± 1.20
Spt1	3.8	3.0	3.4	± 0.40
Spt2	5.3	4.8	5.1	± 0.26
Spt3	4.5	3.8	4.2	± 0.36
Spt4	4.1	3.6	3.8	±0.26

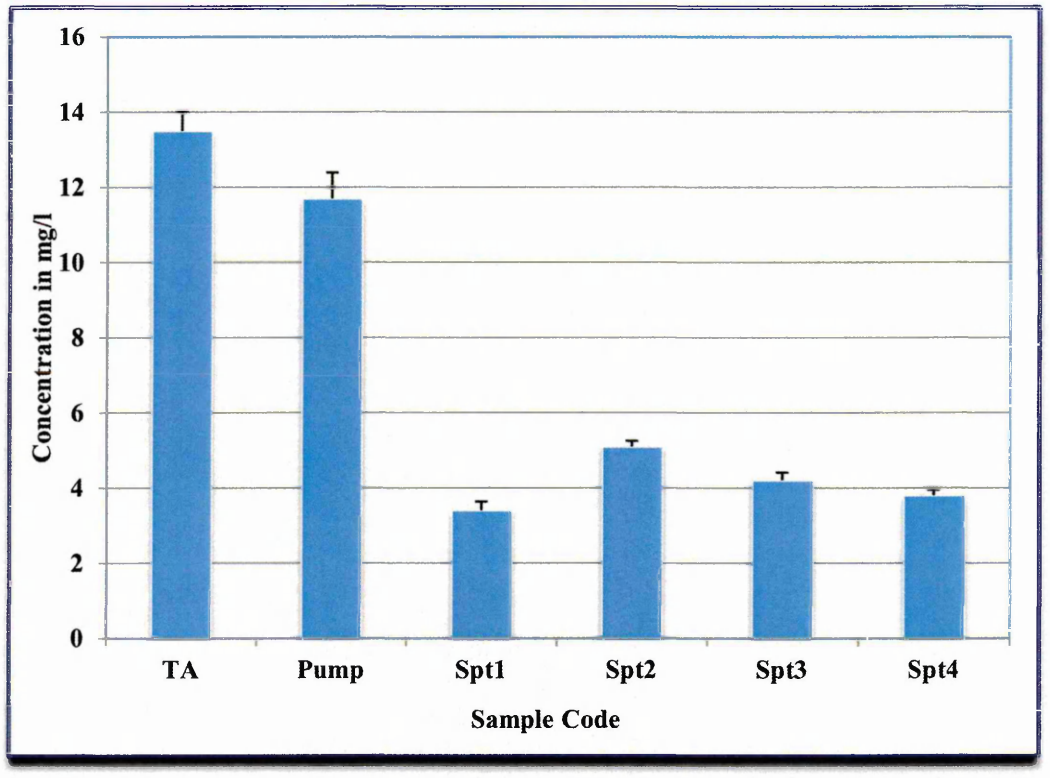
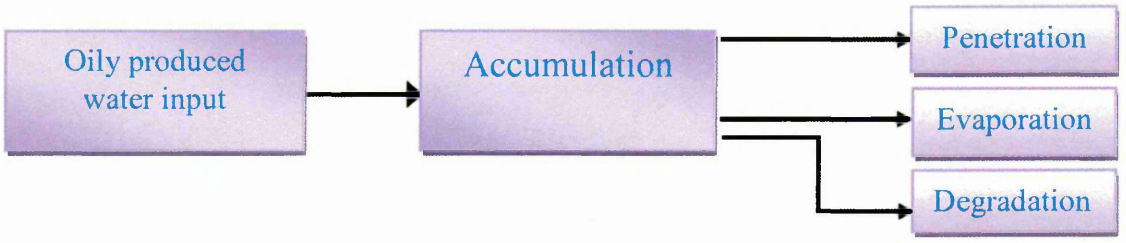


Figure 4-10 Average total extractable hydrocarbons (TEH) concentration in produced water samples

4.3.3 Hydrocarbons analysis in soil

The soil is considered the main affected media since it is in direct contact with the PW. The initial quantity of residual crude oil in the PW is reduced by a number of mechanisms as is shown below. Some will settle or penetrate through the soil at the bottom of the pit edge. Volatile compounds will be removed by evaporation and some

components will be degraded by weathering or endogenous bacteria. However the largest amount of oil will be accumulated in the water bulk of the pit. So that it is very easy to observe the huge quantity of free phase oil accumulated in the water bulk of the pit over the long time of PW disposal. According to field investigations during the site visit, the site is highly polluted by oily PW disposal as shows in Figures 2-3 and 2-4.



In order to find out the contaminant distribution in the area under investigation and to evaluate the soil impact by hydrocarbon, the site was divided into 18 equal sampling points as shown in Figures 2-17 and 2-18, a total of 18 soil samples were collected then the petroleum hydrocarbon (PH) in soil samples were measured. The hydrocarbon content in the soil was determined by TPH-IR, TPH-GC, TSPH and TEH and the results of the total petroleum hydrocarbons using different analytical techniques are presented Table 4-4. The TPH were detected in twenty soil samples from the study area, namely A2 to A6, B1 to B6, C1 to C6, OS (Aged polluted sample) and ReS (Reference soil sample) (the schematic sampling location is shown in Figures 2-17 and 2-18).

The average total petroleum hydrocarbons concentration (TPH-IR) measured for triplicate samples by the IR method ranged from 10550 ± 52 mg/kg at sampling point A6 to 90750 ± 274 mg/kg at sampling point A2 as shown in Figure 4-11. Soil sample A6 was polluted by hydrocarbons approximately nine times less than soil sample A2. The contamination by petroleum hydrocarbons causes serious damage to the soil properties and to the ground water over a long time of disposal of PW into an open environment. The concentrations of TPH for the reference soil sample taken from a location about 300 meters far from the contamination area indicate that the soil is uncontaminated. So the hydrocarbon content of the soil samples shows that the soil around the pits is suffering from high pollution by hydrocarbons, and this is an expected result due to continuous accumulation of crude oil carried by PW, on the soil. Hydrocarbons were not detected in the reference soil samples taken 300 meters from the pit (Figure 4-11).

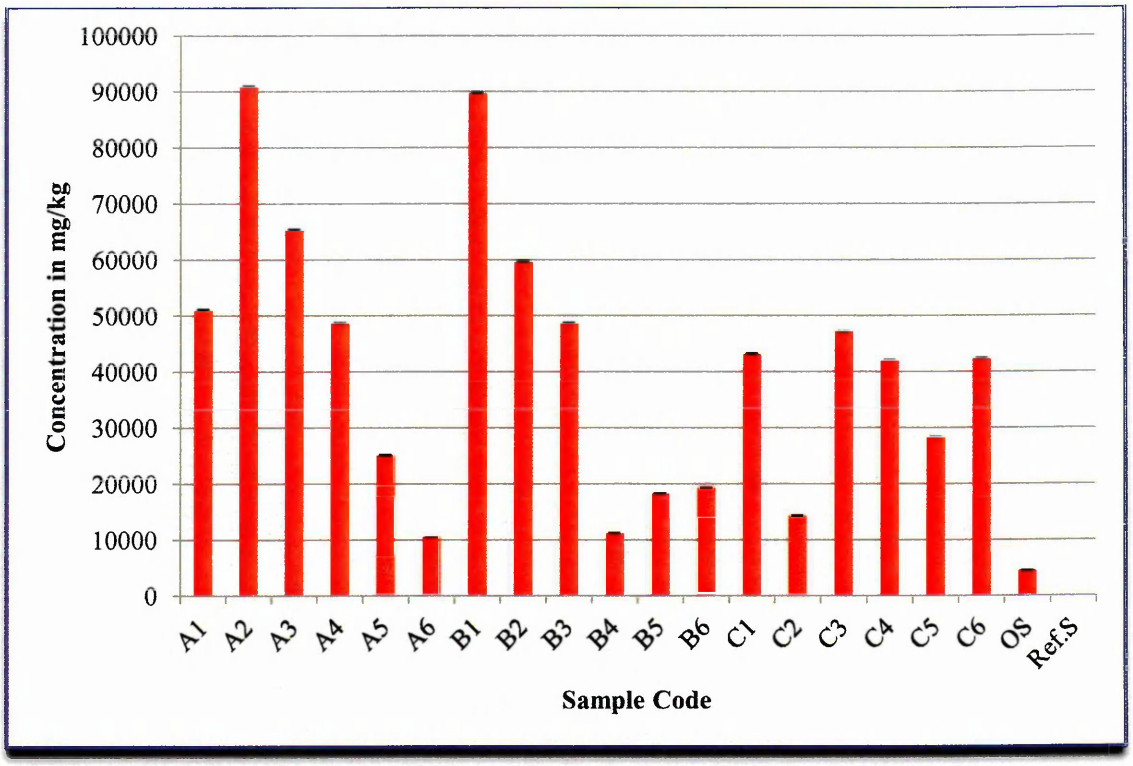


Figure 4-11 Concentration of total petroleum hydrocarbon in soil by IR method

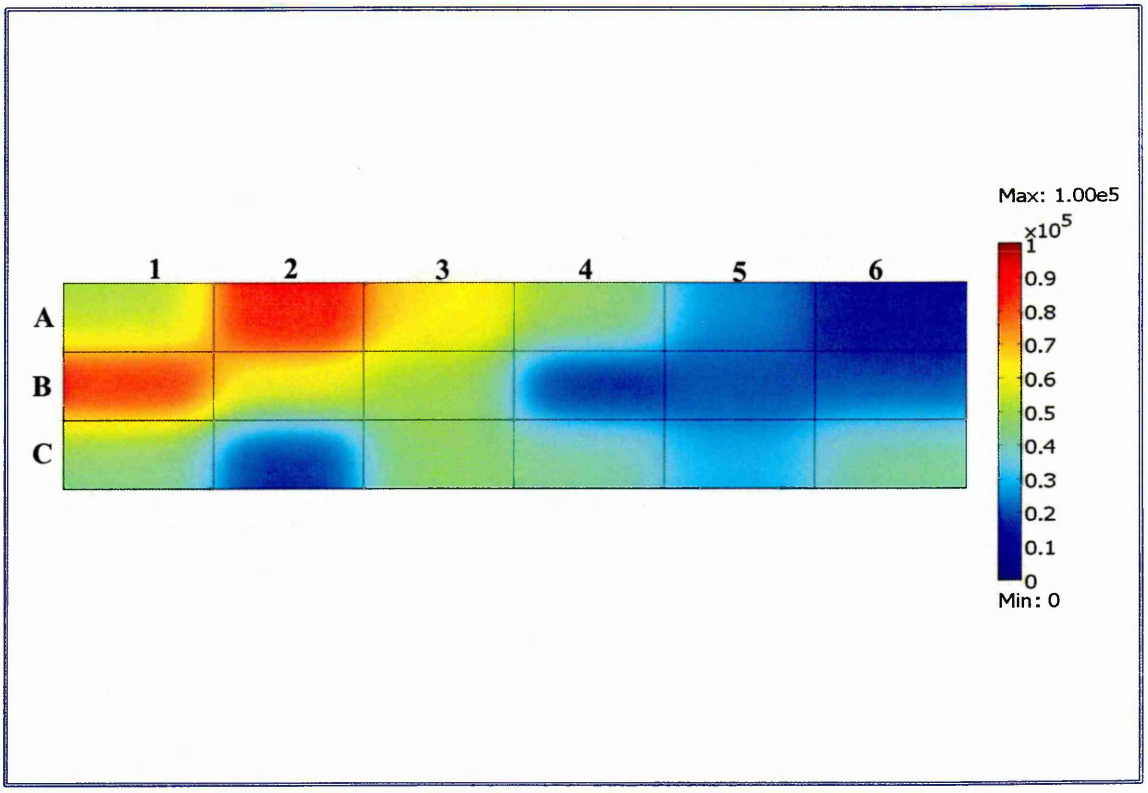


Figure 4-12 Distribution profile of total petroleum hydrocarbons through the surface soil at the bank of produced water pit

The distribution profile of the total petroleum hydrocarbons (TPH) is plotted in Figure 4-12. These data indicates impaction of soil by discharged hydrocarbon associated with

PW (note in the reference soil sample TPH was not detected). Hence it is very easy to observe the huge quantity of oil accumulated in the surface soil around the pits over the long period of oily PW disposal at the Nasser oilfield, Libya as shown in Figure 4-13.

Figure 4-13 Oil accumulated at the surface soil around the disposal pit bank of the Nasser oilfield, Libya

The determination of TPH in samples extracted from contaminated soil or water by GC-FID is usually based on the calibration curve constructed from reference oil. Since the composition of a fresh crude oil and a weathered environmental sample of this crude oil can differ substantially, it is very difficult to choose a proper standard for calibration purposes. The concentration of TPH using GC-FID ranged from 8733 to 72267 mg/kg, this is considered to be very high, while in the reference soil sample TPH was not detected as shown in Figure 4-14. The hydrocarbon concentrations of the soil samples are high, these results clearly indicated that the site is highly polluted with crude oil and this impaction of soil due to the discharged hydrocarbon associated PW. The concentration of TPH by GC method for weathered soil samples was 2558 mg/kg as shown in Figure 4-14 and Table 4-4. Weathering sample refers to the result of biological, chemical and physical processes that can affect the type and the concentration of hydrocarbons that remain in a soil. The concentrations of TPH determined by IR were higher concentrations than those determined by GC. This is because the IR method detects many other organics, as well as petroleum hydrocarbon

including fatty acids which are a very large component of non-petroleum organics (Ololade et al., 2009).

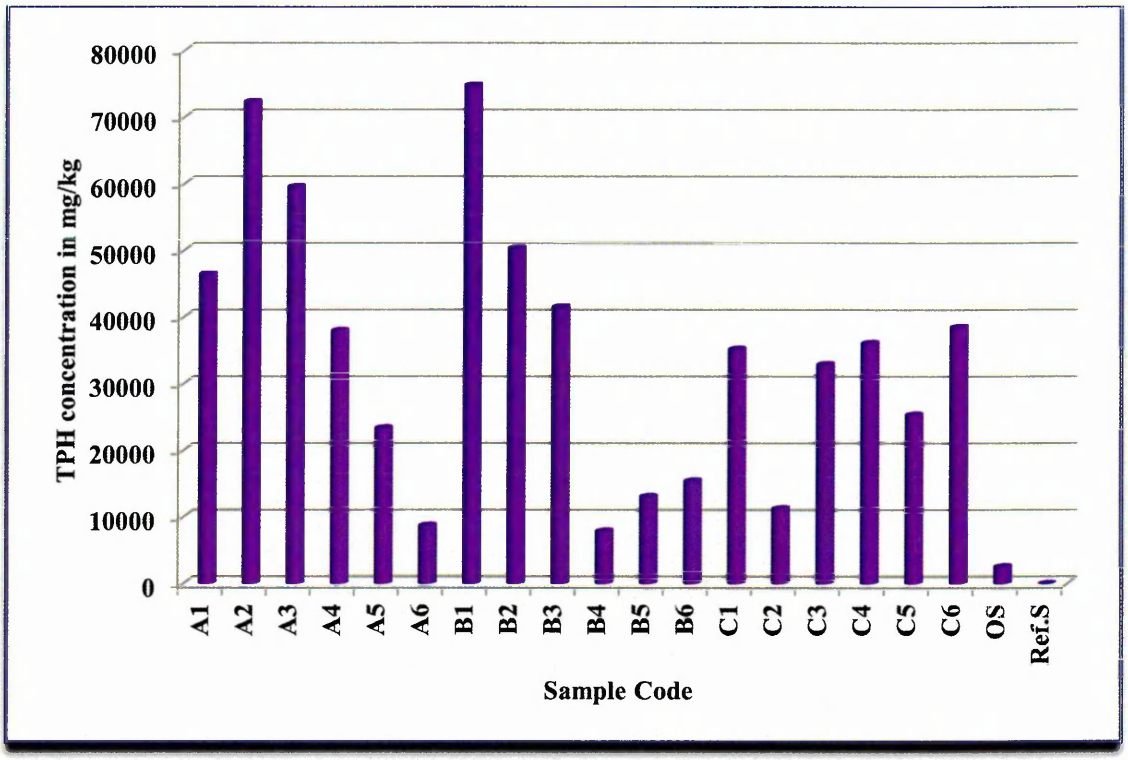


Figure 4-14 Concentration of total petroleum hydrocarbon in soil samples (mg/kg) using GC-FID method

Crude oil contains a complex mixture of compounds which can be categorized into four fractions: saturates, aromatics, resins and asphaltenes. The crude oil was extracted from soil samples using soxhlet extraction which was followed by further fractionation into saturated (aliphatic), aromatic hydrocarbons and resins by glass column chromatography packed with silica gel. The components of interest were eluted with suitable solvents, then after evaporation of the solvent the TSPH eluted from the column were determined gravimetrically, the results are shown in Figure 4-15. The average concentrations of TSPHs ranged from 11028 mg/kg ± 228 to 89777 mg/kg ± 1295 with an overall average in the study area of 39377 mg/kg. The lowest concentration of saturates was observed in sample B4 with an average of 11028 mg/kg ±228, while the highest concentrations was recorded for sample A2 with an average of 89777 mg/kg ± 1295. The level of TSPH was not recordable in the reference soil sample. These data clearly indicate that the soil is highly polluted with hydrocarbons associated with PW in comparison to the result for the reference soil sample as shown in Figure 4-15 and Table 4-4. The concentrations of TSPHs in weathered soil samples (OS, twenty years old polluted soil samples) taken from the dry pit at the Nasser oilfield was 2079 mg/kg dry

weight which was much lower than fresh polluted soil samples as shown in Table 4-4. In general, the concentrations of TSPHs decrease in weathered samples with a relative increase in their aromatic content; this is due to natural biodegradation taking place as well as other physical and chemical process. It is known that saturates are degraded faster than aromatics and others, so that the aromatic content increases in the weathered samples (Gogoi et al., 2003).

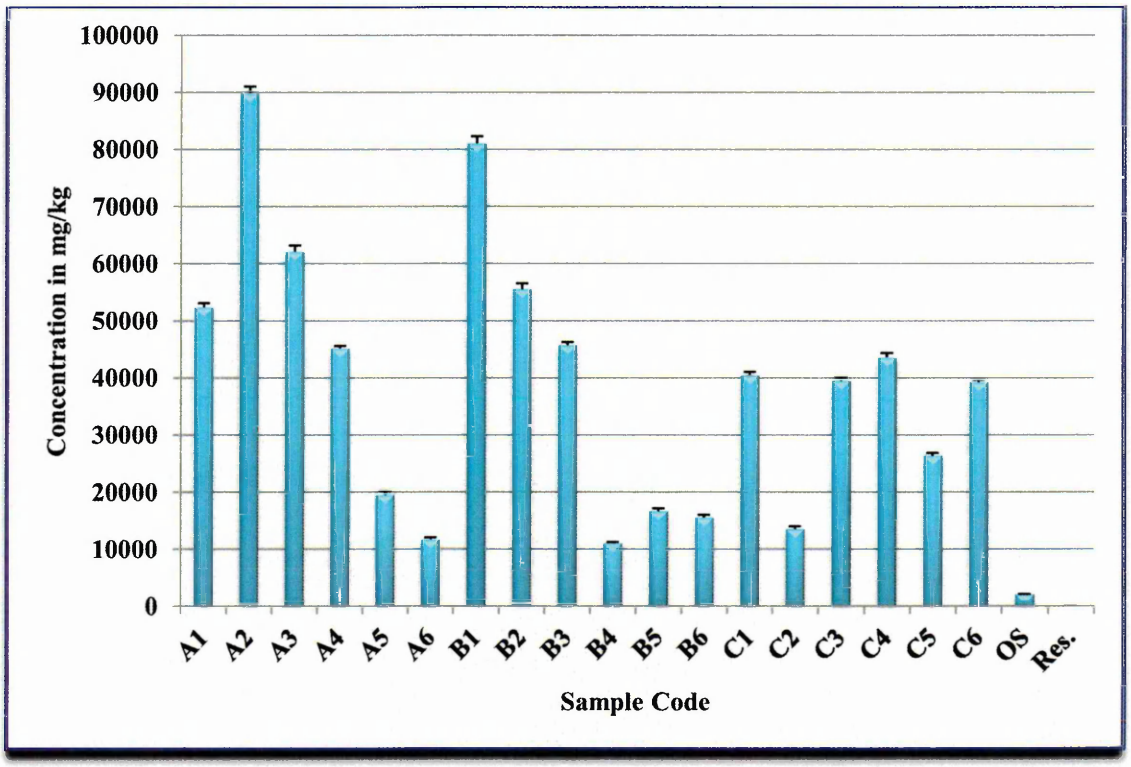


Figure 4-15 Concentration of total saturated petroleum hydrocarbons (TSPH) in soil samples extracted by hexane (gravimetric method)

It is possible to identify hydrocarbon contamination and type in soil or water by extracting the hydrocarbons using a suitable solvent and analysis by GC-FID to determine the carbon distribution. Hydrocarbon contaminated soil samples were extracted by soxhlet extraction with DCM. Then the extracts were then analyzed for TEH by gravimetric analysis after the solvent was evaporated. TEH concentrations in soil samples ranged from 14388 to 134826 mg/kg dry weight. The lowest value was observed at sampling point A6 and the highest value was recorded at sampling point A2. The TEH in soil samples measured by the gravimetric method indicated that these concentrations were significantly higher in comparison to the THE concentration measured for the reference soil sample (438.93 mg/kg) collected from the study site as shown in Figure 4-16. However, the extracted material from reference soil sample was analysis by GC-FID in order to identify the carbon distribution of the contamination and

it was confirmed that the extracted material from the reference soil sample was not petroleum hydrocarbons but it was related to natural organic matter present in soil.

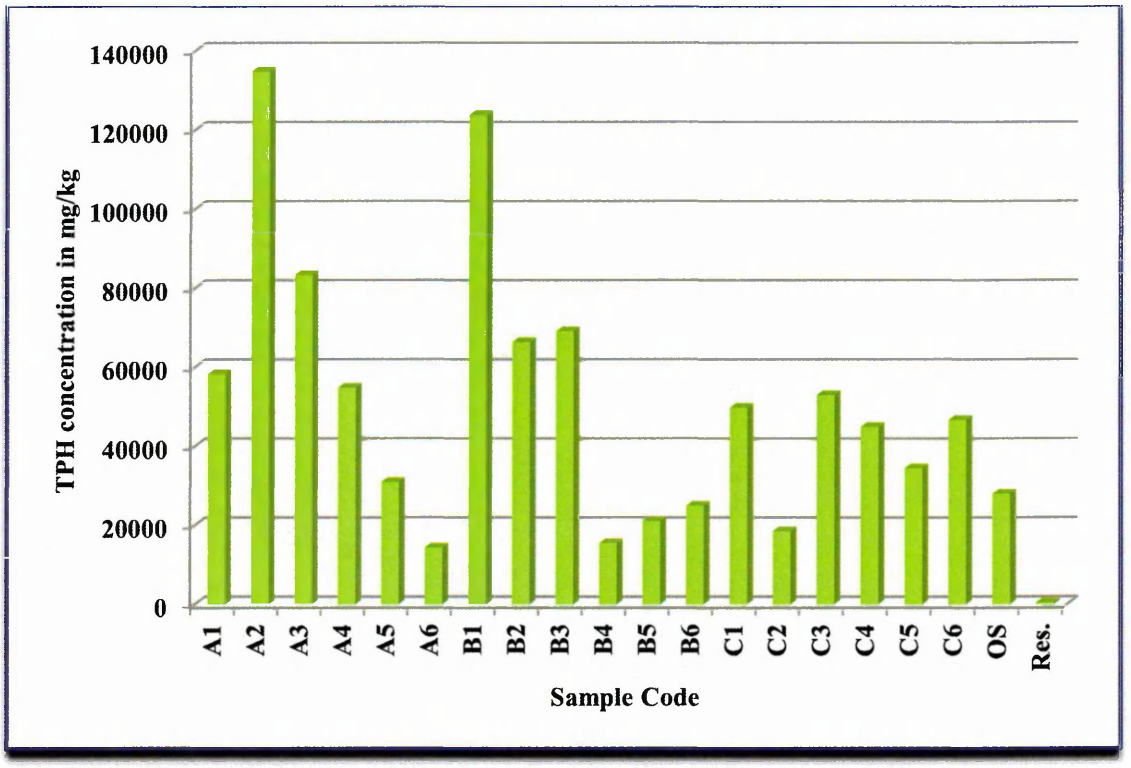


Figure 4-16 Concentration of total extractable hydrocarbons (TEH) in soil samples extracted by dichloromethane (gravimetric method)

The results for TPH are comparable with data obtained by other authors, for instance, Li et al., 2002 reported that the concentration of total petroleum hydrocarbon in soil collected from the Shuguang oil recovery region, Liaohe OilFields in China, ranged from 25800 to 77200 mg/kg dry weight. In addition, Pandiyan et al., 2004 determined TPH in soil collected from an area affected by the Mexican petroleum industry. The concentration was 30810 mg/kg. In another study, Trindade, et al., 2005 measured the concentration of TPH in Brazilian soil affected by four years of contamination and found to contain 54000 mg/kg of total petroleum hydrocarbons.

Das et al., 2007 determined TPH in crude oil polluted soil collected from Oil and Natural Gas Commission of India Oil Well. The concentration reported was 84000 mg/kg. Mukherjee et al., 2007 reported that the concentration of saturated hydrocarbon extracted from a contaminated soil from north east India is 47000 mg/kg. Mancera-Lopez et al., 2008 reported that the concentration of TPH in soil collected from two zones in Veracruz, Mexico. The first is near to a raw oil treatment plant in Poza Rica, Veracruz. The average concentration in soil is 60,600 mg/kg. The second zone is in the marshes of Santa Alejandrina, near the oil refinery in Minatitlan Veracruz, Mexico, the

average concentration is 500,000 mg/kg. These TPH values are within or higher than the concentrations measured in the surface soil of the current study. Furthermore, Liu et al., 2009 have measured the concentration of TPH in contaminated soil collected from the Jidong Oilfield near Bohai Bay, China. This was found to be 52709 mg/kg which is similar to that reported by Xua et al., in 2010 for polluted soil collected from a crude oil spill site in Liaohe Oilfield, Liaoning Province, China, which was found to contain 29500 mg/kg. The previously values of TPH reported within the range or close to those found for surface soil of this study site. But Ramirez et al., 2009 has reported the concentrations measured by gravimetry of TPH extracted from weathered polluted soil sample taken from an oil-contaminated site located in Tabasco, Mexico, as 184377 mg/kg which is much higher than the concentrations found in this study. In the literature, sometimes it is difficult to find data recording (TPH-IR) (TPH-GC), (TSPH) and (TEH) relations which were determined in study site. For comparison, Table 4-4 and Figure 4-17 shows the concentration of total petroleum hydrocarbon determined in the soil samples by four different methods, TPH with IR, TPH with GC/FID quantification [using hexane as the extraction solvent], gravimetric quantification with hexane as extraction solvent and gravimetric quantification with DCM as the extraction solvents. Table 4-4 presents the comparison of the TPH results measured in polluted soil by using different methods (i.e. TPH-IR, GC-TPH and TSPH gravimetric methods). TSPH (gravimetric method) and IR methods give higher TPH concentrations while the GC methods resulted in lower concentrations of TPH as shown in Table 4-4 and Figure 4-17. This suggests that the hydrocarbon values measured by DCM extraction method (TEH) are likely to be overestimated, even higher than those by TPH-IR, GC-TPH and TSPH (gravimetric method), because the (TEH) method extracts the full range of hydrocarbons present in soil, and therefore, it also detects some natural organic materials which are non petroleum hydrocarbons based. The comparison of TPH concentrations in soil between TPH-IR and TPH-GC measurements do not indicate a significant difference except for sample A2 and B1 as shown in Table 4-4. In summary, even though the three currently used TPH methods presented good correlations between them, the IR and TSPH gives higher TPH values and showed no significant differences with the GC method. TEH method gave the highest TPH values and showed significant differences with the other three methods, because the (TEH) method extracts the full range of hydrocarbons present in soil, and therefore, it also detects some natural organic materials which are non petroleum hydrocarbons based. This study shows that TPH contents in soil collected around the pit of the Nasser oilfield were high, and that long-

term disposal of oily PW into the open environment has led to an increased content of TPH on exposed soil. Therefore, it is evident that the extensive crude oil exploitation and production has led to contamination of the soil.

The extracted hydrocarbons from contaminated soil were subject to GC-FID in order to identify the carbon distribution of contaminates. These data are shown in Figures 4-18 to 4-20. The chromatograms show a carbon distribution from C_{11} to C_{34}. Fresh crude oil collected from the production well from the study oilfield was also subject to GC-FID under the same conditions as the samples to be used for comparison. In this case the carbon distributions ranged from C_5 to C_{34}^*. The results for the GC-FID analysis of the extracted hydrocarbons from all soils collected from the study site showed a very slight variation in the carbon distribution as shown in Figures 4-18 to 4-20. The carbon distribution chromatograms display the differences in hydrocarbon fraction extracted from soil samples and fresh crude oil samples. The high molecular weight hydrocarbons are trapped by the soil, where as the low molecular weight hydrocarbons are able to migrate deeper or evaporate to the air. The composition of crude oil released into the environment changes as a result of evaporation, dispersion and degradation. The hydrocarbons extracted from soil samples collected from the Nasser oilfield were seen to be heavily weathered in comparison to fresh crude oil.

Figure 4-21 show the carbon distribution comparison of the overall extracted oil from soil samples with the carbon distribution of fresh crude oil collected from the production well at the Nasser oilfield. As can be seen from Figure 4-21 there is a decrease in the percentage of volatile compounds and an increase in the percentage of semi-volatile and non-volatile compounds. This is due to the weathering condition (volatilization and biodegradation). In general, the saturated hydrocarbons are more easily degraded in the environment compared to their aromatic counterparts. Even in the aromatic fraction, the concentration of mono-aromatics such as BTEX (benzene, toluene, ethylbenzene, and xylene) decreases significantly due to weathering (volatilization and biodegradation). In the case of PAHs, the concentrations of volatile PAHs such as naphthalene decreases while the concentration of 4 and higher ring PAHs increases (Wang et al., 1995 and Sun et al., 2011).

Table 4-4 Hydrocarbons found in soil samples by different methods (in mg/kg)

Sample Code	TPH-IR Method	TPH-GC-FID Method	TSPH by hexane (Gravimetric Method)	TEH by DCM (Gravimetric Method)
A1	50908±429	46440	52368±755	58280
A2	90750±274	72267	89777±1295	134827
A3	65213±205	59540	62129±1067	83347
A4	48616±138	37963	45180±433	54853
A5	24998±115	23378	19498±747	30991
A6	10550±52	8733	11678±337	14388
B1	89678±194	74760	81053±1222	123813
B2	59590±160	50343	55557±963	66371
B3	48620±127	41507	45723±564	69127
B4	11123±86	7898	11028±228	15545
B5	18215±115	13087	16666±482	21117
B6	19324±99	15422	15561±482	24994
C1	43121±126	35204	40432±629	49752
C2	14300±47	11269	13523±486	18533
C3	47140±113	32899	39493±665	52960
C4	41990±111	36099	43559±807	44926
C5	28280±59	25361	26360 ±500	34491
C6	42262±149	38484	39201±469	46696
OS	4500	2558	2079.21	27987
Re.S	n.d	n.d	n.d	439

n.d= not detected

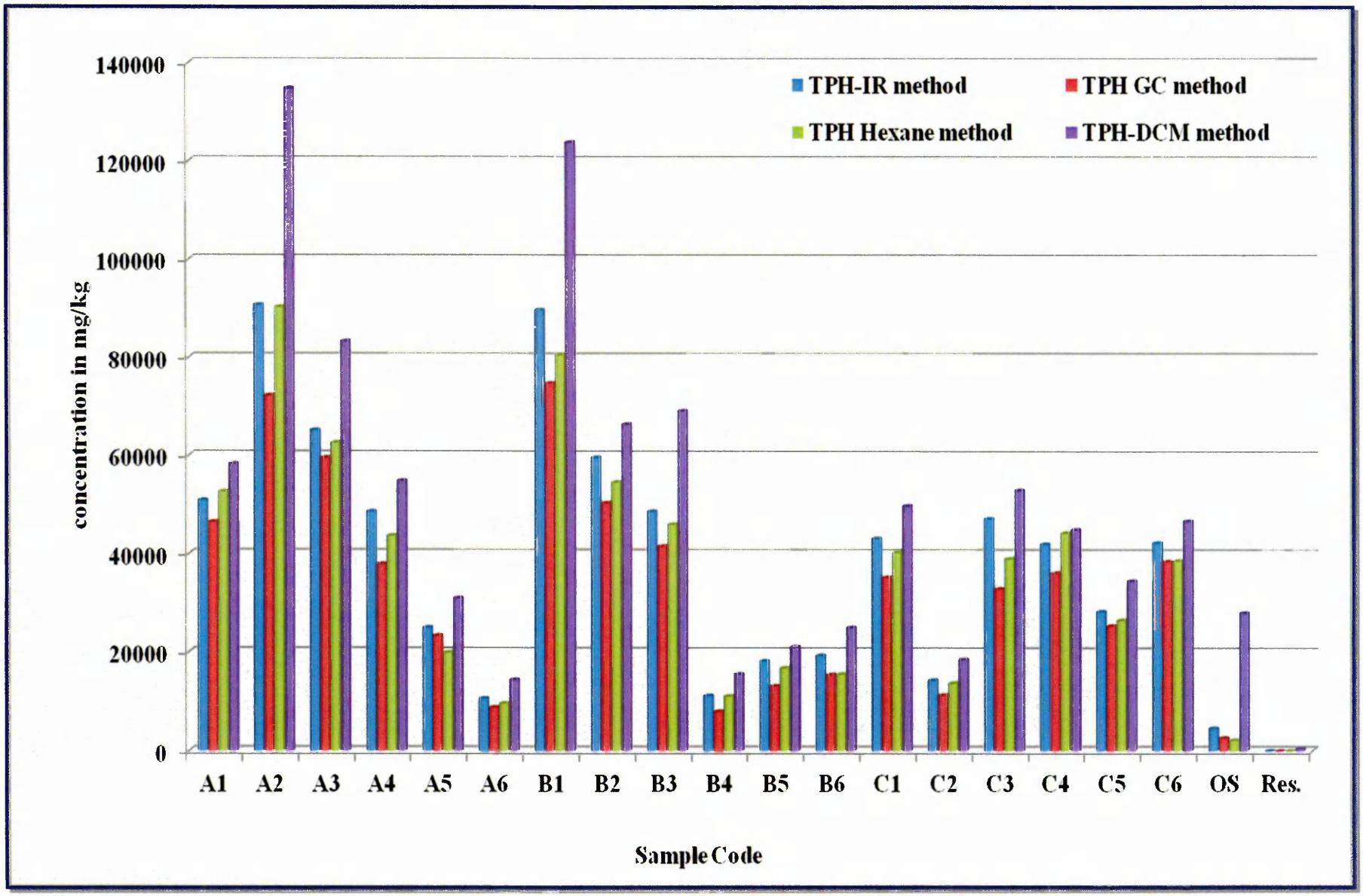


Figure 4-17 Comparison of the total petroleum hydrocarbons (TPH) concentrations in soil using different analytical methods

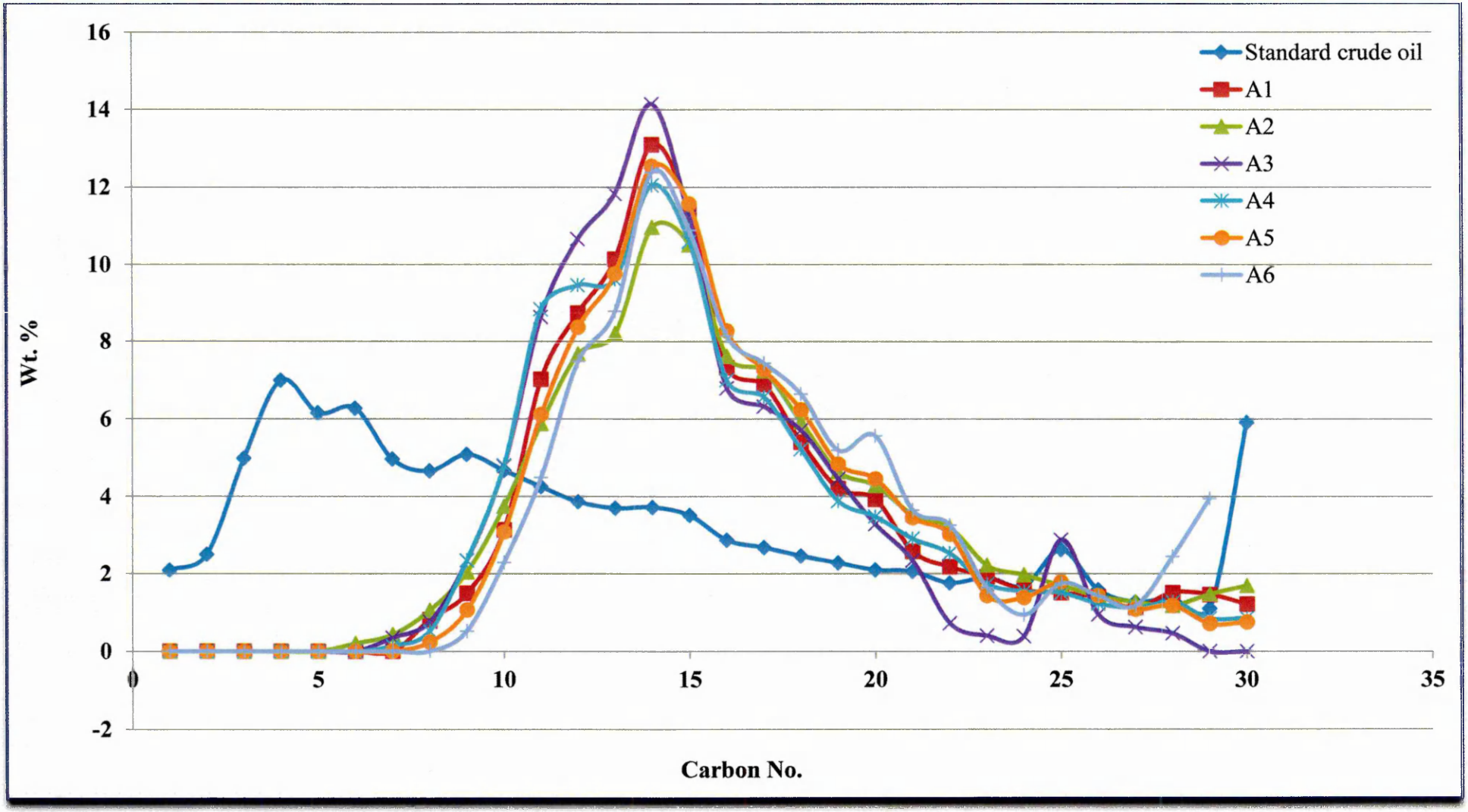


Figure 4-18 Carbon distribution comparison of hydrocarbons extracted from soil samples and standard crude oil

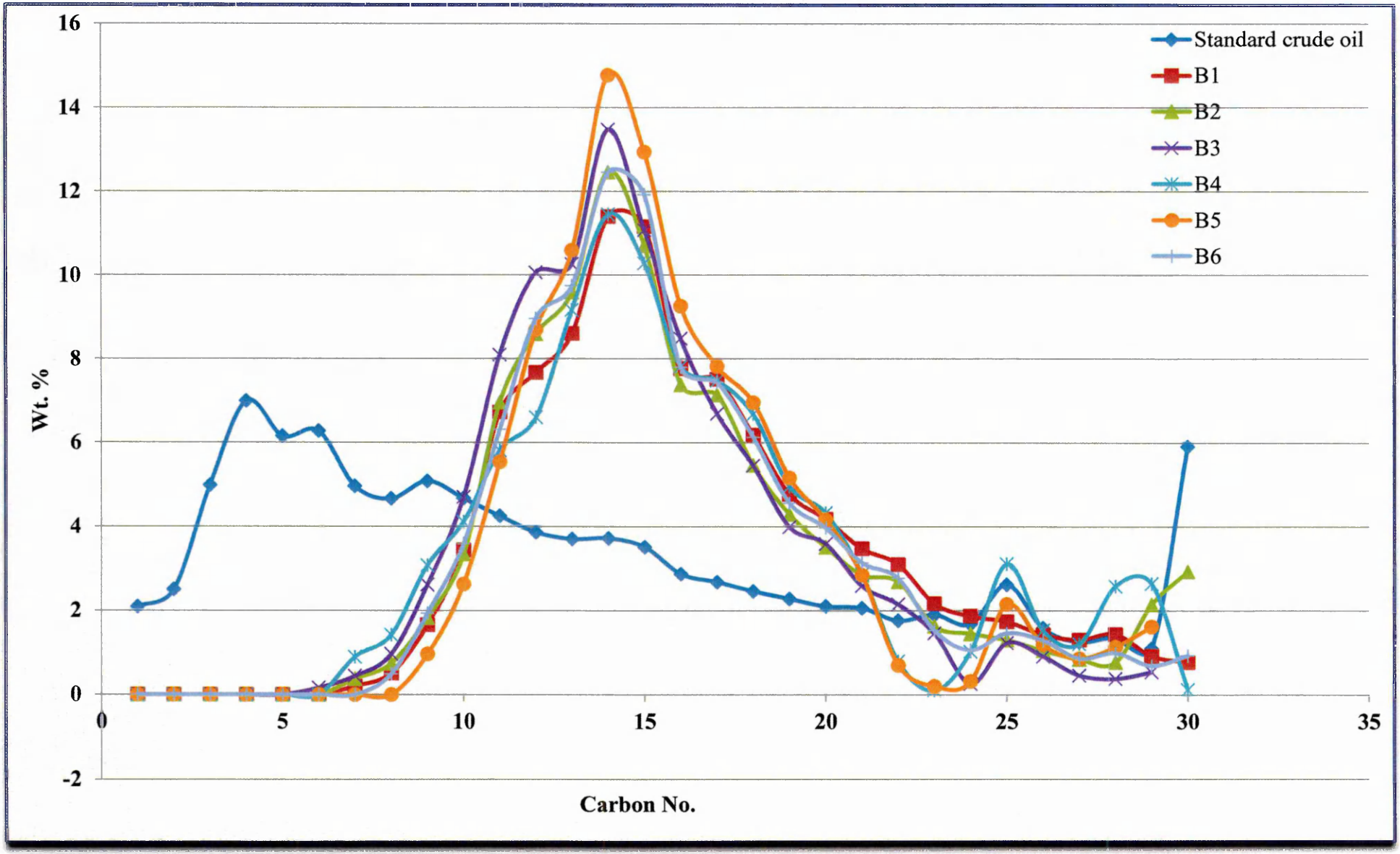


Figure 4-19 Carbon distribution comparison of hydrocarbon extracted from soil samples and standard crude oil

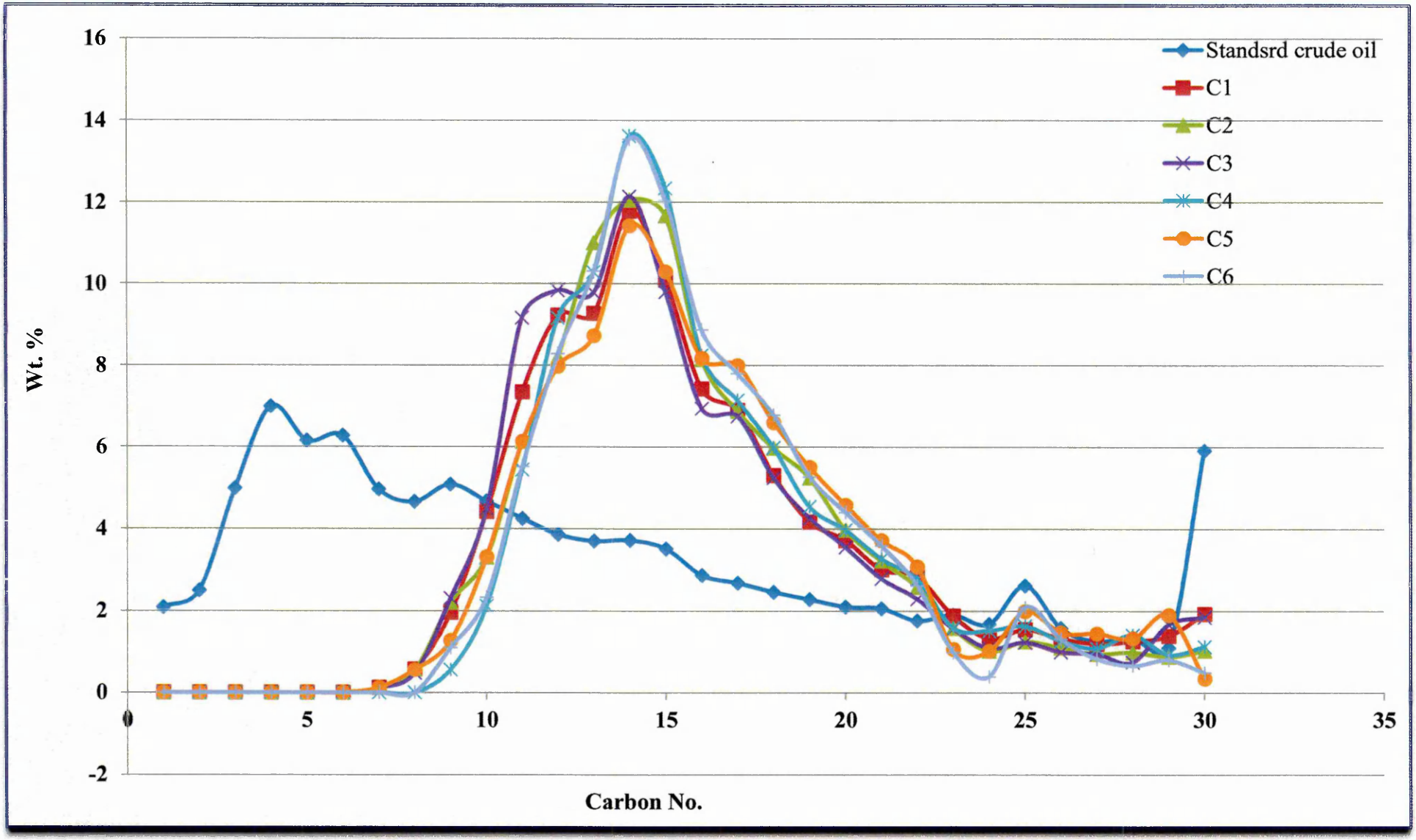


Figure 4-20 Carbon distribution comparison of hydrocarbon extracted from soil samples and standard crude oil

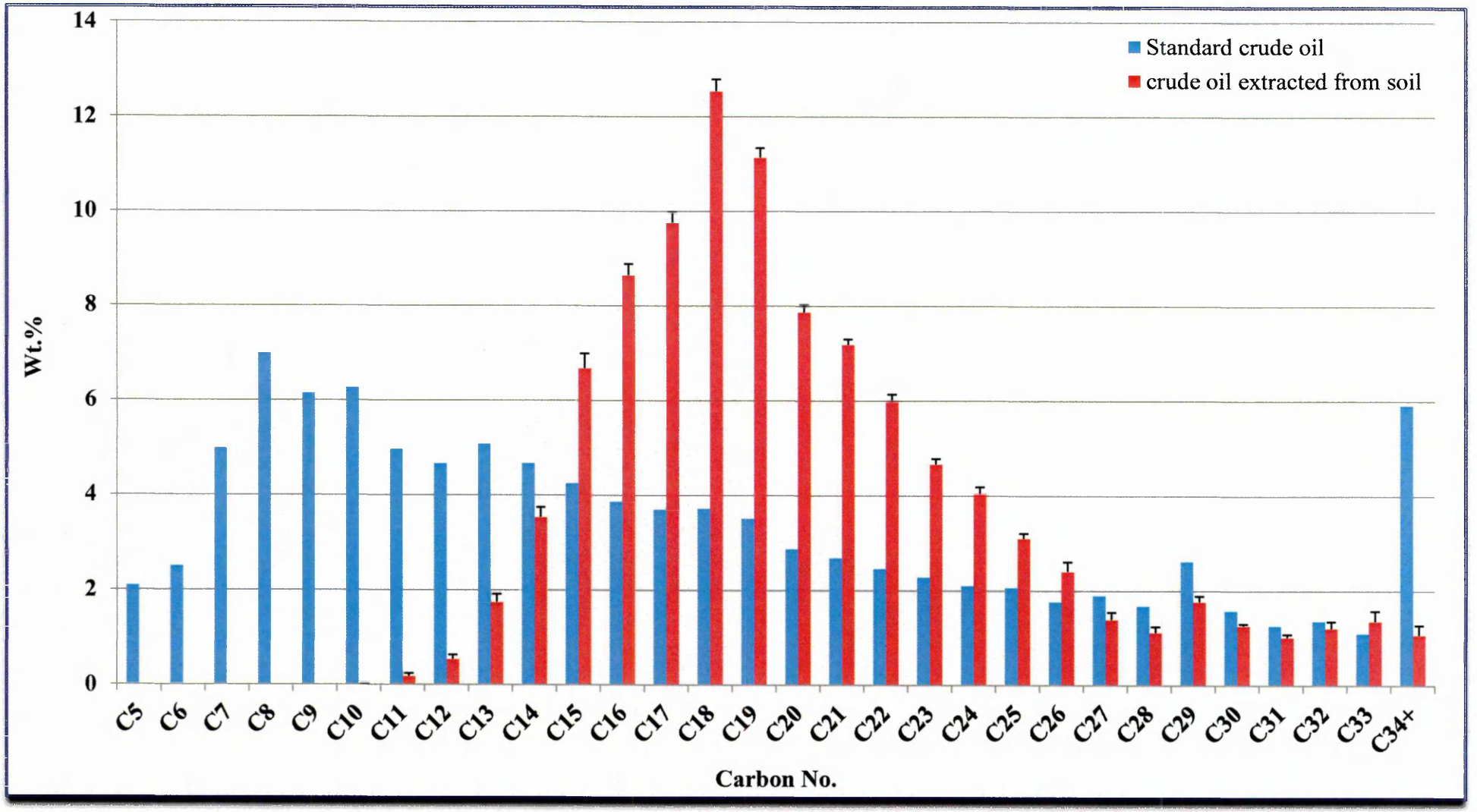


Figure 4-21 Carbon distribution comparison of hydrocarbon extracted from soil with standard crude oil collected from the Nasser oilfield

4.3.4 BTEX analysis in produced water

As it is mentioned above, PW contains large amounts of various hazardous organic compounds such as monoaromatic hydrocarbon benzene, toluene, ethylbenzene, ortho-, meta, and para-xylenes (i.e. BTEX) which are not strongly adsorbed by soil and are relatively highly soluble in water when compared to the other hydrocarbons, such as aliphatic hydrocarbons and aromatic hydrocarbons (AlSalka et al., 2010 and AlSalka et al., 2011). BTEX are toxic and the most dangerous compound is benzene, since it is recognized as a carcinogen and has an adverse effect to human health and the environment. Contamination of water by these compounds is a very serious problem (AlSalka et al., 2010 and AlSalka et al., 2011). Many methods have been used to measure BTEX in soil and water including GC coupled with multiple techniques such as, purge and trap, static headspace (HS), and headspace SPME. In recent years, a number of direct sampling mass spectrometric methods have been developed for the analysis of environmental samples including solvent free sample preparation methods. SPME, liquid phase microextraction (LPME) and direct aqueous injection (DAI), these have been developed as an alternative to traditional methods for sample preparation to eliminate time of operation, the large volume of solvent used for extraction and the use of specialized tools (AlSalka et al., 2010). BTEX were used as target analytes in this investigation using SPME-GC-FID, since they are common PW pollutants. Table 4-5 shows some properties of BTEX. To calibrate the SPME-GC-FID system an external standard was established, that is, serial dilutions of BTEX standard solution were prepared and each standard was measured as described previously.

Six point calibrations (based on peak areas) were obtained over the concentration range of interest. Linear calibration curves were obtained for each of BTEX compounds. The correlation coefficients ranged from 0.9944 to 0.9994 as shown in Figure 4-7. Thus, the analytical calibration curves were used for quantification of each BTEX in PW samples. Analysis of BTEX in PW samples collected from the area of the Nasser oilfield was achieved by using SPME coupled with GC-FID. For the SPME analysis, first the analytes were sorbed on to a fibre and then desorbed from the fibre to the GC inlet. The proposed method was used to quantify BTEX in PW. The SPME was operated under the optimum conditions. Triplicate analyses were performed in selected PW samples. SPME method was applied for the determination of BTEX in PW samples collected from the study site under the optimum conditions. The results in Table 4-6 show a summary of the occurrence and the concentrations of target compound in PW samples and clearly show that BTEX was present in PW.

Table 4-5 Standard BTEX properties

Compound	Molecular Weight (g/mol)	Density (g/cm^3)	Structure
Benzene	78.11	0.8787	C_6H_6
Toluene	92.14	0.8669	C_7H_8
Ethylbenzene	106.16	0.867	C_8H_{10}
p+m-Xylene	106.17	0.861-0.88	C_8H_{10}
o-Xylene	106.17	0.861-0.88	C_8H_{10}

Table 4-6 Concentrations of BTEX in PW samples from the Nasser oilfield

Sample Code	Concentration in mg/l					
	Benzene	**Toluene**	**Ethylbenzene**	**p+m-Xylene**	**o-Xylene**	**Total BTEX**
TA	1.38±0.12	0.39±0.012	0.020±0.009	0.04±0.008	0.03±0.008	1.86
Pump	1.31±0.10	0.34±0.028	0.013±0.007	0.02±0.008	0.03±0.008	1.70
Spt1	0.049	0.072	0.0033	0.010	0.0093	0.15
Spt2	0.078	0.107	0.0038	0.016	0.0159	0.22
Spt3	0.035	0.056	0.0019	0.011	0.0102	0.11
Spt4	0.021	0.087	0.0025	0.0095	0.0069	0.13

The values shown in Table 4-6 vary according to the sampling points. BTEX values are much higher, (1.86 and 1.70 mg/l), at storage tank outlet (TA,) and discharged stream (pump) respectively, where the PW is in the earlier steps, before discharging to the pits. In the pit samples (Spt1, Spt2, Spt3 and Spt4), the values range between 0.11 to 0.22

mg/l, and are much lower due to volatilization and cracking reactions which are catalyzed by sunlight and heat. In general, PW taken from the pit contained lower BTEX concentrations than those from the areas close to oil installations (i.e. facilities). It is clear from Figure 4-22 that the highest amounts of BTEX were found in samples taken from the production facilities.

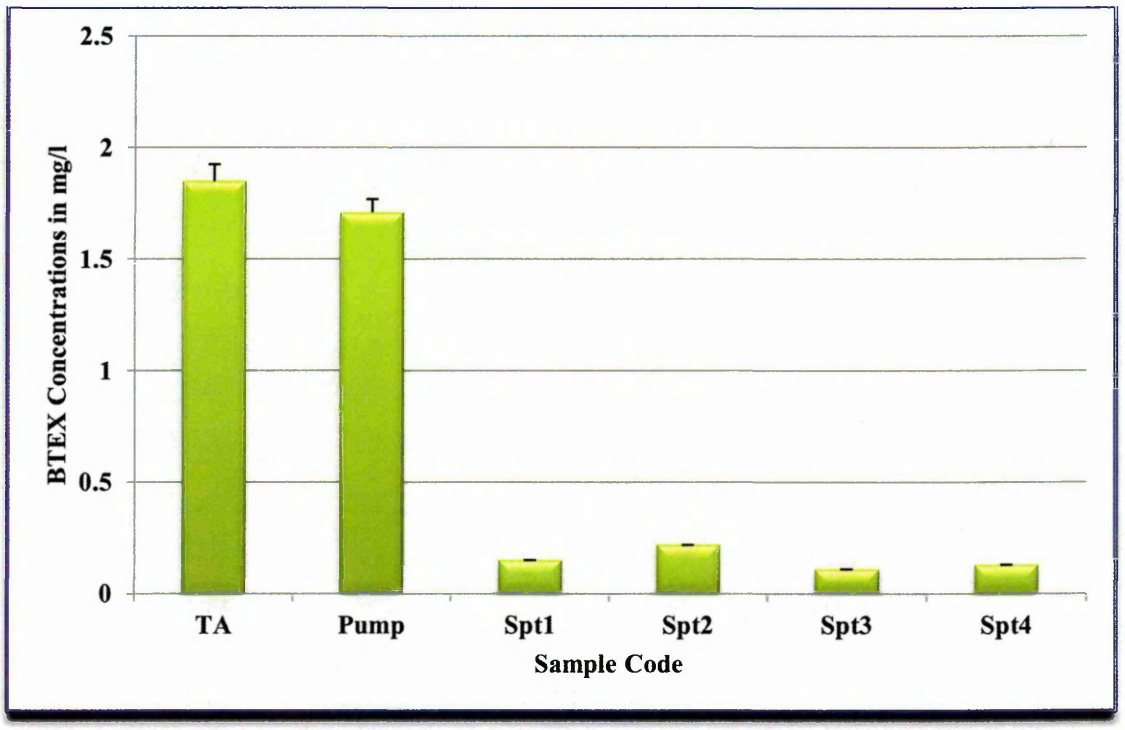


Figure 4-22 Totals BTEX concentration in PW from the Nasser oilfield, Libya

The average concentrations of BTEX in PW ranged from 0.11 mg/l to 1.86 mg/l as shown in Figure 4-22. Concentrations of benzene ranged from 0.021 mg/l to 1.38 mg/l. High concentrations of benzene were observed in the location of TA, where the PW is held before discharging to the pits. A lower amount of benzene was detected at the pit samples Spt4, due to volatilization and cracking reactions which are catalyzed by sunlight and heat. Sampling locations TA and Pump show average concentrations of toluene of 0.39 mg/l ± 0.012 and 0.34 mg/l ± 0.028 respectively (Table 4-6). There is considerable decrease in concentration of the toluene in the disposal pit of the Nasser oilfield at sampling locations i.e. Spt1, Spt2 Spt3 and Spt4 (0.056 to 0.107 mg/l), toluene had the highest concentrations found for BTEX compounds at pit location. ethylbenzene concentrations at sampling locations TA and Pump were 0.020 mg/l ± 0.009 and 0.013 mg/l ± 0.007 respectively. The concentrations of ethylbenzene at the sampling locations Spt1, Spt2, Spt3 and Spt4 ranged between 0.0019 mg/l and 0.0038 mg/l. Lower concentration of ethylbenzene was observed at sampling point Spt3. The highest concentrations of p-m xylene and o-xylene 0.04 mg/l ± 0.008 and 0.03 mg/l ±

0.008 respectively were observed at sampling location TA, while the lowest concentration of p-m xylene and o-xylene 0.0095 mg/l and 0.0069 mg/l respectively, were detected at sampling point Spt4 as shows in Table 4-6. In general the highest concentrations of total BTEX at the study site were observed at the outlet of storage tank (TA), where the PW held before discharging to the pits. The lowest total BTEX was detected in the pit due to volatilization and cracking reactions which are catalyzed by sunlight and heat. However, the levels recorded in this study for the total BTEX in PW are in fact much lower than those found in some other locations. The effluent of (PW) from the Bonsucesso treatment plant located in the city of Carmopolis which is the most important oil and gas producer in the State of Sergipe, North-east of Brazil has been found to have a total BTEX 3.12 mg/l, benzene was 1397 µg/l for toluene was 1263 µg /l, ethylbenzene 148 µg /l, m+p xylenes 216 µg /l and for o-xylenes 96 µg /l (Dorea, et. al., 2007). In the Statoil operated platforms Statfjord B and Gullfaks C which are located in the area of the North Sea the concentration of BTEX were 8900 µg/l and 8350 µg/l respectively (Faksness et al., 2004). Utvik (1999) has reported that the concentrations of total BTEX in PW from North Sea Norwegian oil production fields Osberg, Brage and Troll ranged from 5.8 to 9.0 mg/l, for benzene 3.7 to 4.5 mg/l, toluene 1.5 to 3.5 mg/l, ethylbenzene 0.3 to 0.6 mg/l and the combined concentrations of xylenes 0.2 to 0.7 mg/l (Utvik 1999). Jones R.J. and Heyward have reported that the concentrations of total BTEX in PW collected from the platform of Harrlet oilfield on the Australian mainland was 33.86 mg/l. The concentrations of individual compounds were benzene 2.0 mg/l. Toluene 30 mg/l, ethylbenzene 0.16 mg/l and the combined concentrations of xylenes 1.7 mg/l (Jones, et al., 2003). All of these values are much higher than the levels measured in this study. In addition, Al-Salka et al., 2011 reported significant concentrations of BTEX in PW samples from the Dier Azzor area, Syria. They found benzene 1.16 mg/l, Toluene 0.995 mg/l, ethylbenzene 0.131 mg/l, m+p-xylenes 0.623 mg/l and o-xylene 0.36 mg/l to give a total BTEX concentration of 3.27 mg/l. This is also higher than level measured in this study. Furthermore, Tellez et al., 2002 who found the concentrations of BTEX in PW taken from oil separation facilities, located in south western US within the mineral rich Permian Basin Lea County, New Mexico in the average of 7.7 ±2.0 mg/l. The GC chromatograms for BTEX extracted from the PW samples collected from the study site are given in Appendix C1 to C6, for samples TA, Pump, and Spt1 to Spt4. The peaks in the chromatograms are clearly seen and were identified separately as benzene, toluene, ethylbeneze, p-m xylene and o-xylene according to the retention time of each compound.

4.3.5 Determination of polynuclear aromatic hydrocarbons (PAHs)

PAHs are the most toxic components of petroleum. PAHs are found in environments as a result of industrial effluents (Anyakora et al., 2006 and Yang et al., 2011). PAHs were measured in the PW using an established method. The extraction efficiency of PAHs from soils and sediments might be influenced by several factors, such as soil moisture, polarity of solvents used, the PAH content in samples, and the texture of the soils. PAHs are traditionally extracted from various matrices by soxhlet extraction. In these investigations, liquid-liquid extraction, soxhlet extraction and SPE extraction methods were used to extract the PAH from the sample and followed by analysis with gas chromatography mass spectrometry to measure the concentrations of PAHs. Following the extraction and cleanup procedures described previously. As there were no previously published data on the PAHs contamination of the soil and PW from the Nasser oilfield, Libya a major aim of the present work was to measure PAHs in soil and PW. A total of eight selected soil samples and four PW samples collected from the contaminated pit at the Nasser oilfield were analysis for the 15 individual PAH. The 15 individual PAH compounds analysed in the soil and PW samples included the following: NAP) Naphthalene, ACL) Acenaphthylene, AC) Acenaphthene, FL) Fluorine, PHE) Phenanthrene, AN) Anthracene, FA) Fluoranthene, PY) Pyrene, BaA) Benz(a)anthracene, CHR) Chrysene, BbFA) Benzo(b)fluoranthene, BaP) Benzo(a)pyrene, IP) indeno (1,2,3-cd) pyrene, DBahA) Dibenz(a,h)anthracene and BghiP) Benzo(g,h,i)perylene. Table 4-1 shows the PAHs considered in this study. PAHs were determined by GC-MS with selected ion mode (GC-MS-SIM).

4.3.5.1 Characteristics of PAH standards

The chromatogram indicated in Figure 4-23 shows a representative GC-MS-SIM chromatogram of a standard mixture solution (i.e.15 ppm). All PAHs were satisfactorily separated with adequate sensitivity, where the retention times and peak area for each individual PAH compounds were identify in order to be used in measured the concentration of each PAH compound in the unknown sample. Identification of PAHs was verified by comparisons of the retention times and mass spectra of the PAHs sample with those of standards. Quantification was performed by integration of the selected ion chromatograms. Ions used for quantification are shown in Figure 4-24, these chromatograms are very clear with no interfering peaks appearing in the areas of interest. For quantification, a calibration graph was produced for each standard PAH with the correlation coefficient ranging from 0.9735 to 0.9996 as shown in Figure 4-8.

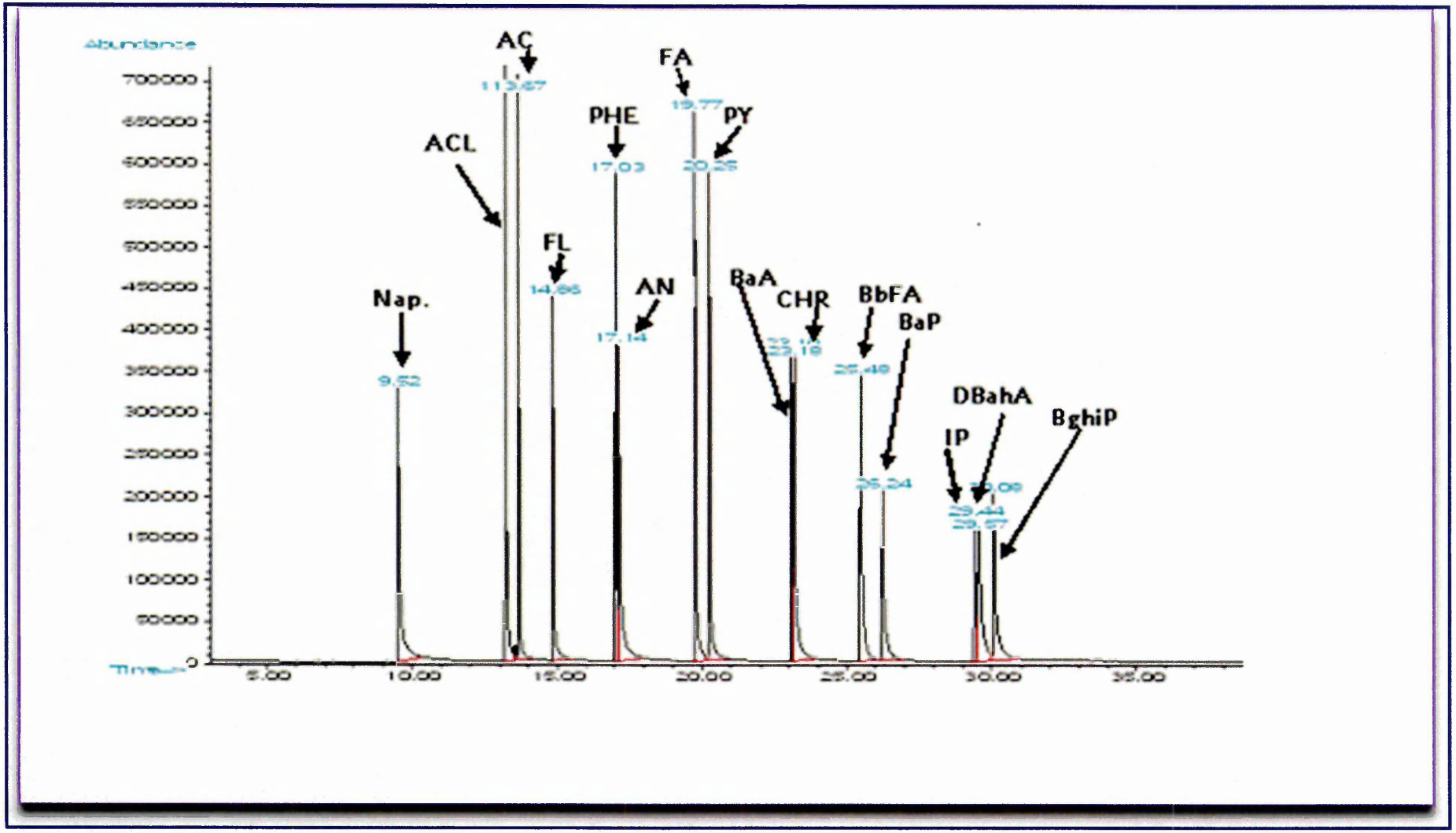


Figure 4-23 GC-MS (full scan) chromatogram obtained for mixed 15 PAHs stock standards solution

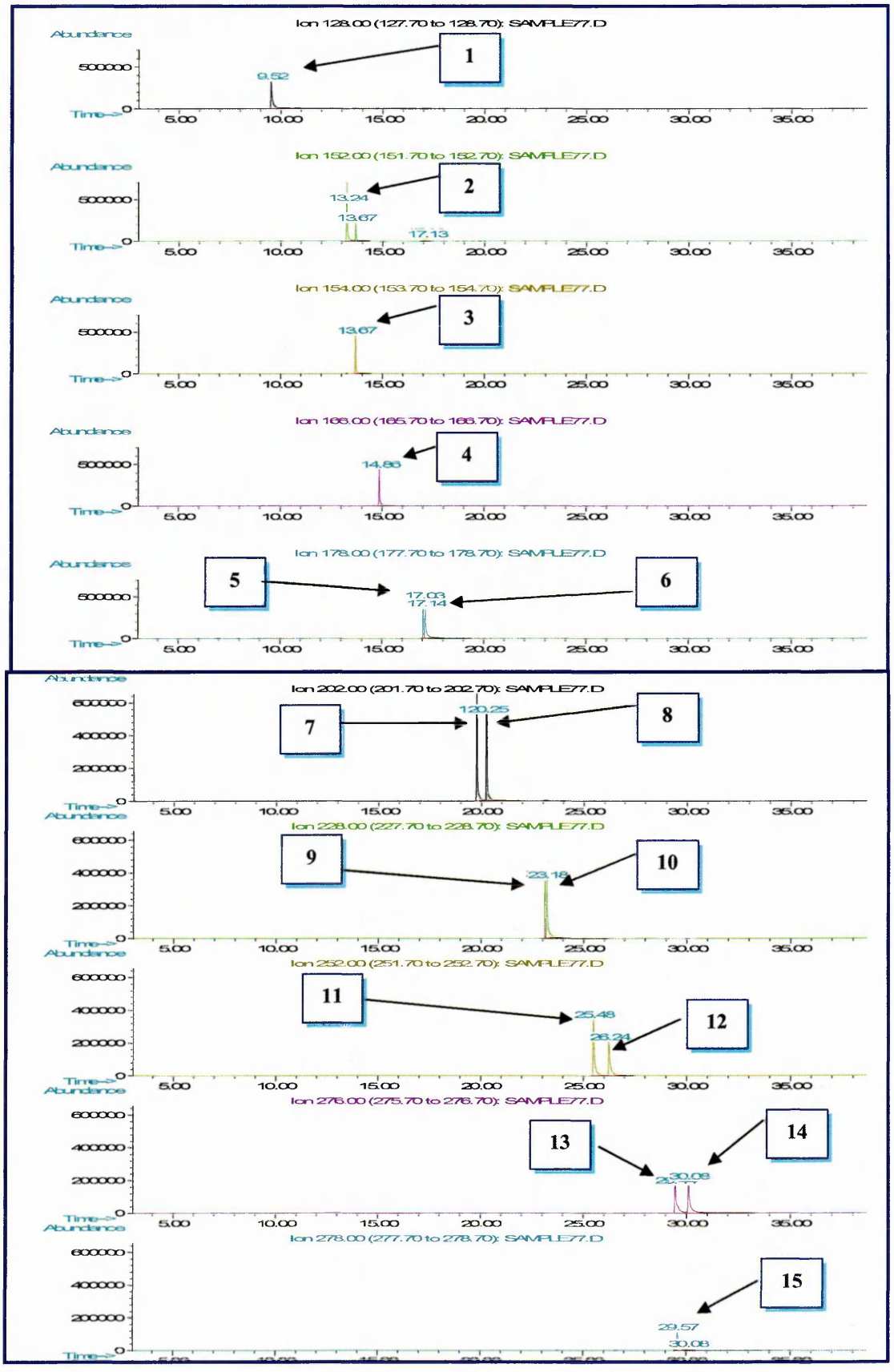


Figure 4-24 Selected ion chromatogram of mixed 15 PAHs standard solution obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP

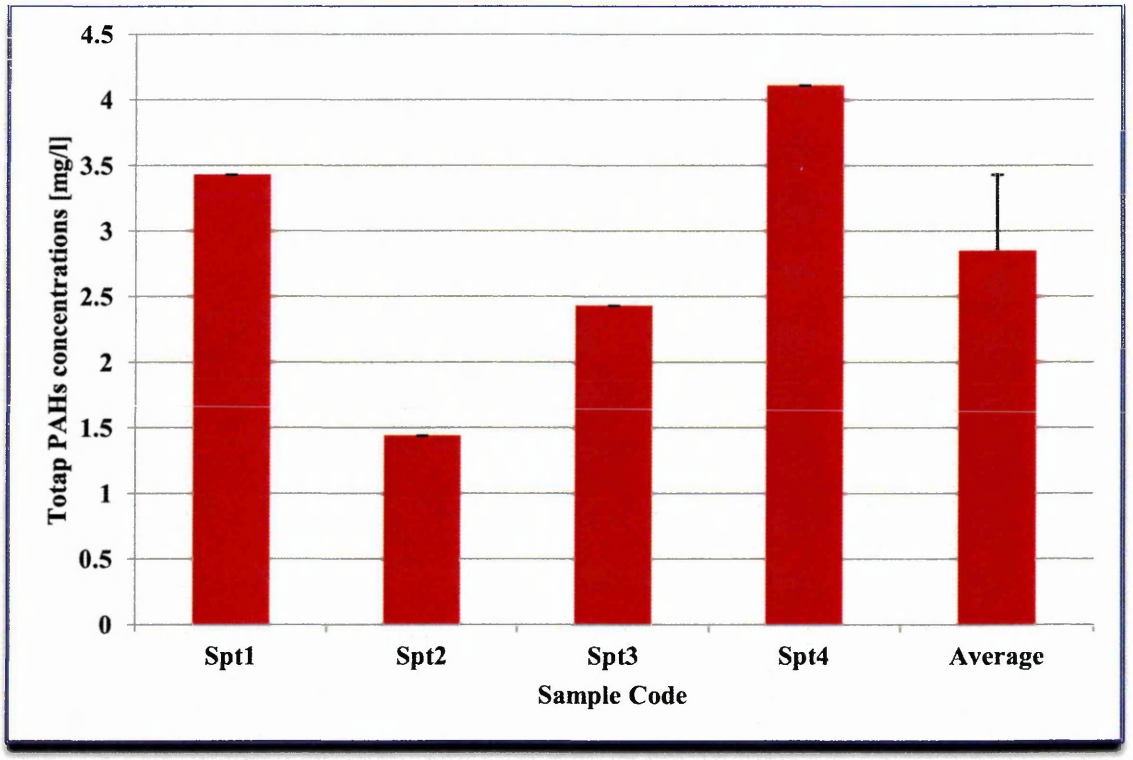


Figure 4-25 Total PAHs concentrations in produced water sample obtained from the pit at the Nasser oilfield area

4.3.5.2 PAH concentration in produced water samples

The total concentration of fifteen PAHs in produced water (PW) samples of four sampling point from the pit at the Nasser oilfield were measured. The PAH determined ranged from 2 to 5 ring compounds with molecular weights from 128 to 278 m/z. The total concentration of PAHs (∑PAH) was calculated by summing the concentrations of 15 individual PAH compounds. The concentrations of PAHS (ΣPAH) in PW at different sampling points are presented in Table 4-7 and Figure 4-25. In the present study the chemical characterisation of the extracts showed that PW samples (Spt1, Spt2, Spt3 and Spt4) collected from the pit at the Nasser oilfield contained ∑ PAH between 1.44 to 4.11 mg/kg with an average of 2.85 mg/kg ± 0.58 as shown in Table 4-7 and Figure 4-25. It can be seen from Figure 4-25 that the highest concentration of ∑PAHs was detected at PW sample Spt4 and the lowest ∑PAHs concentration was detected in PW sample Spt2 collected from the study area. The individual PAH in PW, some of the compounds analyzed were detected at all sampling points and some compounds were not detected such as naphthalene, acenaphthylene and acenaphthene. Different sampling points were detected of different compounds, varying from the low molecular weight to the high molecular weight. This is suggesting that the PW in the area was contaminated by PAHs. The highest concentration of individual PAH level was recorded (0.56 mg/l) for pyrene at the sampling point Spt1. The lowest concentration for indvidual PAH was

detected (0.08 mg/l) for dibenz(a,h)anthracene (Spt2) and some of 15 PAH were undetectable in PW samples. In addition benzo(b)fluoranthene and benzo(a)pyrene were detected in two sampling points out of four. In general the high molecular mass PAHs such as indeno(1,2,3-cd) pyrene, benzo(g,h,i)perylene and dibenz(a,h)anthracene have low solubility in water.

The results of the distribution of PAHs in the PW are presented in Figure 4-26 and Table 4-7. It is evident from the chromatograms (Appendix D1 to D7) that a significant amount of PAHs are present in PW. The obtained results indicated that the PW discharged from the Nasser oilfield to the open environment was contaminated with PAHs and the concentrations of PAHs varied among sampling location. Due to the low levels of PAHs in the PW but high volumes of water discharged an impact on the environment is likely. It is clear that PW results in an input of PAHs to the environment at the Nasser oilfield, Libya. Therefore, living in the Nasser oilfield increases the health hazard of people and is an issue for the ecosystem. A comparison of the present study to those of other oil exploration and production site around the world is shown in Table 4-8. The published studies showing in Table 4-8 revealed that the level of PAHs in PW in the current study are within or relatively higher than those detected in PW samples from other parts of the world and this is may be these compounds are presence attached to dissolved solid or adsorbed on particulates and solubilised in any oily contaminant in PW.

Table 4-7 Concentration and distribution of PAHs (mg/l) in produced water sample obtained from the Nasser oilfield

PAHs	Concentrations of PAH in soil [mg/l]					
	Spt1	**Spt2**	**Spt3**	**Spt4**	**Average**	**STDEV**
NAP	n.d	n.d	n.d	n.d	n.d	n.d
ACL	n.d	n.d	n.d	n.d	n.d	n.d
AC	n.d	n.d	n.d	n.d	n.d	n.d
FL	0.29	0	0.15	0.49	0.23	0.10
PHE	0.53	0.13	0.41	0.49	0.39	0.09
AN	0.27	0.13	0.27	0.48	0.29	0.07
FA	0.46	0.26	0.37	0.6	0.42	0.07
PY	0.56	0.27	0.47	0.48	0.45	0.06
BaA	0.27	0.14	0.2	0.36	0.24	0.05
CHR	0.24	0.17	0.19	0.28	0.22	0.02
BbFA	0.12	n.d	n.d	0.14	0.06	0.04
BaP	0.2	n.d	n.d	0.25	0.11	0.07
IP	0.21	0.14	0.17	0.24	0.19	0.02
BghiP	0.15	0.12	0.1	0.14	0.13	0.01
DBahA	0.13	0.08	0.1	0.16	0.12	0.02
ΣPAH	3.43	1.44	2.43	4.11	2.85	0. 58

n.d = not detected

Table 4-8 PAHs levels in produced water around the world

Origin	Concentration (mg/kg)	Reference
Libya	1.44-4.11	Present Study
North Sea, 1997	0.94-1.37	Utvik et al., 1999
North Sea, 2004	1.14-1.18	Faksness et al., 2004
Bonsucesso treatment plant, State of Sergipe, Brazil, 2007	0.037 (11 PAHs only)	Dorea et al., 2007
City of Carmopolis, Sergipe, Brazil, 2011	0.18	Bispo et al., 2011
Syria Dier Azzor area, 2011	0.35	Al-Salka et al., 2011
Treatment plant Nigeria, 2008	0.83	Okoro 2008
discharges from Norwegian Oil 2005	0.80-10.93	Hawboldt et al., 2005
Tampen regions of Norwegian Oil of the North Sea, 1998	0.49-1.99	Neff et al., 2006
discharges from UK Oil, 2005	0.01-0.86	Hawboldt et al., 2005
discharges from Dutch Oil, 2005	0.34-7.2	Hawboldt et al., 2005

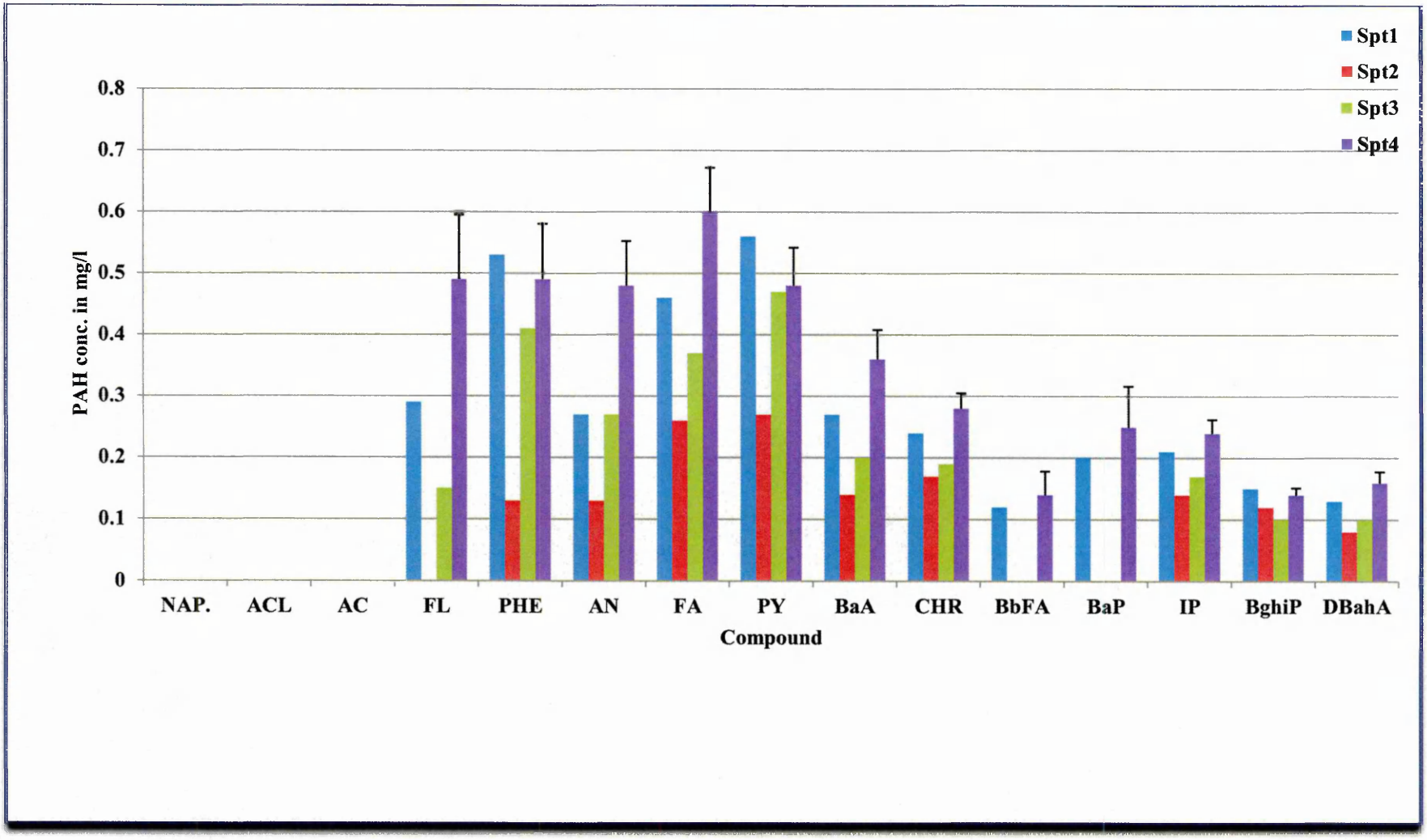


Figure 4-26 PAH distribution in PW samples collected from the study area

4.3.5.3 PAH concentration in soil samples

Soil and sediment are the major sinks for PAHs, primarily because of the low solubility of these compounds and their strong affinity for organic carbon in particulate matter, PAHs can remain in the soil for many years. Gas chromatography conditions were set to give a baseline separation of the target compounds in a reasonable time. This was achieved by setting the chromatographic conditions as described previously. Calibration curves were obtained using a series of varying concentrations of standard containing each of the 15 PAHs. For quantification, the curves were obtained by plotting the standard peak area against the standard concentrations used. A linear regression function was set up on calibration curves with correlation coefficient from 0.9727 to 0.9994. Furthermore, identification of PAHs was confirmed by comparisons of the retention times and mass spectra of the PAHs sample with those of standards. Quantification was performed by integration of the selected ion chromatogram extracted from total ion current. Ions used for quantification are shown in Figures 4-23 and 4-24. The PAHs concentrations in soil were determined by using GC-MS in selected ion monitoring mode under the same condition as the standards solution.

The results obtained from the study site for total PAHs (Σ PAH) and the individual PAHs are presented in Table 4-9. The PAHs were detected in eight soil samples selected from the study area, namely A2, A4, A6, B3, B4 C1, C4 and C6 (the schematic sampling location was shows in Figures 2-17 and 2-18). Total PAH, defined as the sum of the concentrations of the 15 PAHs that were detected in a sample. The PAHs concentrations measured in soil samples collected from the study site are under three categories: heavily contaminated soil with crude oil; moderately contaminated soil with crude oil; and slightly contaminated soil with crude oil. The total concentrations of 15PAH (Σ PAH) in soil samples ranged from 11.73 to 40.94 mg/kg dry weight. The total PAH (Σ PAH) concentrations of soil sample A2 was significantly greater by approximately four times than soil sample A6. The highest PAHs (Σ PAH) concentrations (40.94 mg/kg) were observed at the sampling point A2, because this sample is heavily polluted with crude oil. As a result, the high concentrations of PAHs in soil could be caused in changes in the soil type and by the large amount of PW discharged. Some of these PAHs will penetrate to the ground water through the soil column. Therefore, PAHs determination in soil may provide important information in the state of environmental pollution of an area of study, and measurement of PAH concentrations is important in establishing basic information for future monitoring and assessment of PAHs accumulation in the surrounding areas of the Nasser oilfield.

The soil samples A4, C1 and C4 seem to be contaminated at the same degree since their PAHs concentrations range from 20.92 to 25.55 mg/kg with an average of 23.16 mg/kg ± 2.32. The PAHs concentrations in soil samples B3, B4 and C6 ranged from 16.73 to 18.45 mg/kg with an average of 17.69 mg/kg ± 0.86. Soil sample A6 had the lowest PAH concentration as shown in Table 4-9 and Figure 4-27. It can be seen clearly from Table 4-9 and Figure 4-27 that the sample point (A2) contained most of the PAH compounds and that these were in high concentrations in comparison to the other soil samples analyzed. The total PAH concentrations decreased significantly with decreasing hydrocarbon-impact soil from 40.94 mg/kg in heavily contaminated soil sample A2 to 11.73 mg/kg in low contaminated soil sample A6. The overall average of total PAH (ΣPAH) concentration in the Nasser oilfield soil samples was 21.87 mg/kg ± 8.77 (n=8) as shown in Figure 4-27.

Most compounds analyzed were detected at all the sampling point the concentrations of the individual PAH ranged from 0.11 to 8.04 mg/kg. Naphthalene (NAP) is a very common PAH found at petroleum and its derivates polluted sites. Naphthalene (NAP) is a 2-ring compound and considered by USEPA as a priority pollutant (Iturbe et al., 2005). This compound was not detected at all the sampling points of the study site. Acenaphthylene (ACL) is a 3-ring compound and is often found in petroleum and its derivatives. In this study ACL was detected in all analyzed soil samples except sampling point A6. The concentrations ranged from n.d to 0.67 mg/kg. Low molecular weight PAHs, such as ACL, were detected in highest concentrations at heavily polluted soil sample A2, while the lower concentrations in polluted soil sample A6 was not detected. Acenaphthene (AC) was found at the study site in the range of 0.12– 1.48 mg kg. AC is a 3-ring compound and it is not carcinogenic. Fluorene (FL) is a 3-ring PAH and not considered as carcinogenic compound (Iturbe et al., 2005 and Kerr et al.., 2001). This compound was present in soil collected from the study site at concentrations between 0.14 to 2.72 mg/kg. It can seen from Table 4-9 and Figure 4-28 that the highest individual PAH level were recorded for phenanthrene (PHE) in all eight soil samples analyzed, in addition the PHE was detected at greatest levels in the heavily oil contaminated soil sample A2. Generally, the detection frequency of PAHs decreased with decreasing hydrocarbon concentration as it is seen in soil sample A6 as shows in Table 4-9. The concentrations of PHE ranged between 3.04 to 8.04 mg/kg. Phenanthrene (PHE) is a 4-ring PAH and reported as non carcinogenic compounds. Anthracene (AN) was found at a concentration range of 0.74–2.18 mg/kg in eight soil samples. This PAH compound is a 3-ring and non-carcinogenic compound.

Fluoranthene (FA) was detected in all sampling points at a concentration ranging from 0.80 to 2.88 mg/ kg. This compound is 4-ring PAH and has been reported as carcinogenic (Iturbe et al., 2005). The concentrations of pyrene (PY) were measured in soil samples and ranged from 1.01 to 3.29 mg/kg. Benzo(a)anthracene (BaA) is a 4-ring compound has been reported by Iturbe, et al., 2005 as a carcinogenic compound. BaA was detected in soil samples at study area and ranged from 0.90 to 3.72 mg/kg. Chrysene (CHR) also detected in all sampling point at concentrations ranging from 1.01 to 2.83 mg/kg. Chrysene (CHR) is 4-ring compound and is reported as carcinogenic. Benzo(b)fluoranthene (BbFA) is a 5-ring PAH and considered as carcinogenic. BbFA was identified in soil samples; the range of concentrations for this compound was 0.32 to 1.72 mg/kg. Benzo(a)pyrene (BaP) is 5-ring PAH and also considered carcinogenic compounds and was identified in all sampling points at the Nasser Oilfield, Libya. The concentrations of benzo(a)pyrene (BaP) ranged from 0.75 – 2.99 mg kg. Finally, indeno(1,2,3c-d)pyrene (IP), benzo(g,h,i)perylene (BghiP) and dibenzo(a,h)anthracene (DBahA) are 5-ring PAH compounds and have been classified as possible carcinogens (Anyakora et al., 2006 and Morillo et al., 2007), these compounds were detected in soil samples, the concentrations ranged from 0.38 to 3.06 mg/kg, 0.46 to 2.7 mg/kg and 0.29 to 2.66 mg/kg respectively as shown in Table 4-9.

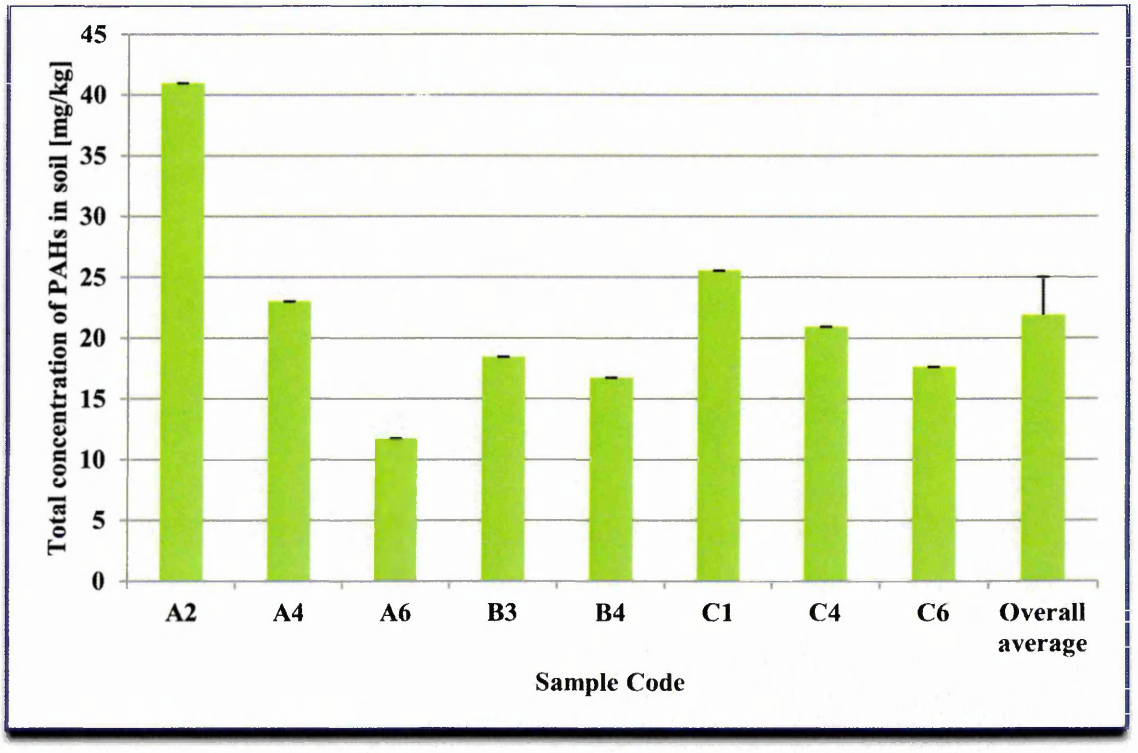


Figure 4-27 Total PAHs (Σ PAH) concentrations in the polluted soil samples from the Nasser oilfield

Table 4-9 Concentration and distribution of 15 PAHs in polluted soil samples obtained from the Nasser oilfield, Libya

PAHs	Concentrations of PAH in soil [mg/kg]							
	A2	A4	A6	B3	B4	C1	C4	C6
NAP	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
ACL	0.67	0.30	n.d	0.16	0.14	0.11	0.21	0.25
AC	1.48	1.34	0.15	0.27	0.40	0.13	0.47	0.12
FL	2.72	1.34	0.14	0.98	0.68	1.68	0.90	0.85
PHE	8.04	6.70	3.20	5.18	3.06	6.11	5.63	3.75
AN	2.18	1.38	1.46	1.55	0.74	1.33	2.11	1.42
FA	2.88	1.59	1.03	0.80	1.16	1.72	1.61	1.33
PY	3.29	1.60	1.01	1.54	1.42	2.30	1.81	1.44
BaA	3.72	1.75	1.32	3.16	1.50	1.41	0.90	2.32
CHR	2.83	1.64	1.01	1.32	1.63	1.70	1.72	0.86
BbFA	1.72	0.59	0.32	0.57	0.91	1.20	0.63	0.50
BaP	2.99	1.36	0.96	0.75	1.43	1.58	1.87	1.17
IP	3.06	1.17	0.38	0.88	1.29	2.42	1.20	1.25
BghiP	2.70	1.20	0.46	0.90	1.17	2.34	1.02	1.29
DBahA	2.66	1.04	0.29	0.39	1.2	1.52	0.84	1.06
ΣPAH	40.94	23.00	11.73	18.45	16.73	25.55	20.92	17.61

n.d = not detected

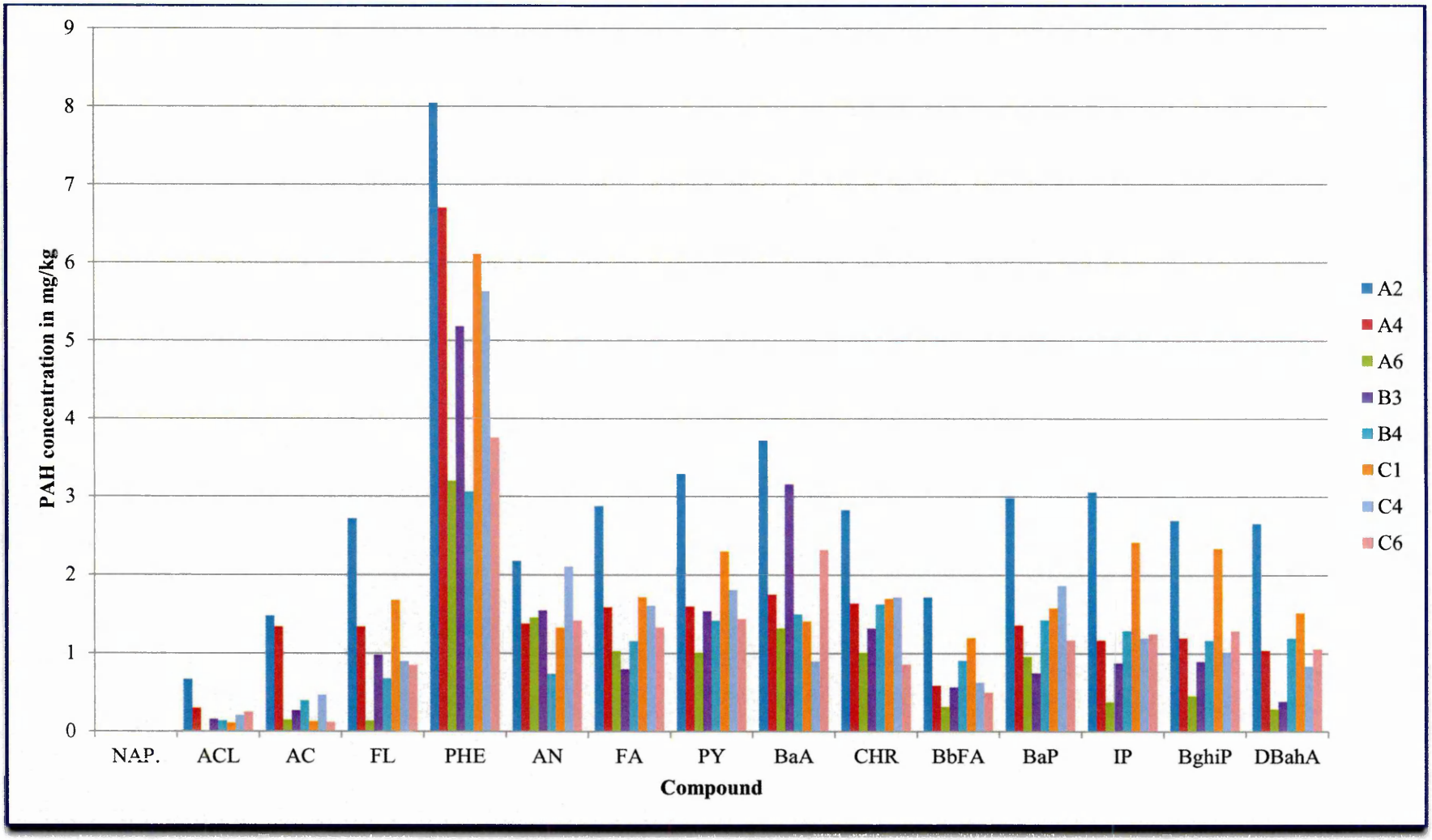


Figure 4-28 PAH distribution in soil samples from the Nasser oilfield

In comparison, Bojes et al., 2007, reported the PAHs concentration in soil contaminated with oil collected from oil exploration and production sites located in environmentally sensitive and geographically distinct areas throughout Texas. These ranged from 31.0 mg/kg to 86.4 mg/kg. The highest levels of PAH were found in heavily polluted soil samples were 86.4 mg/kg dry weight and the lower PAHs concentration were 72.5 and 31.0 mg/kg in moderately and slightly contaminated soils, respectively,. These values are much higher than this investigation. The soil samples from the eight sampling points showed different degrees of PAH contaminations that were much higher than the PW samples. The reason for this is probably that PAHs are generally insoluble in water but can be readily solubilised in organic acid. This mean that in aqueous environment they are found adsorbed on particulates and solubilised in any oily contaminant that may be present in water. Overall, the results of this investigation indicate elevated concentrations of PAHs in soil samples around the disposal pit of the Nasser oilfield caused by the disposal of oily PW. The total PAH concentrations measured in soil of the study area (Table 4-9 and Figure 4-28) are much lower than those found in some other polluted soil samples in other locations around the world. Table 4-10 represents a comparsion of the PAHs concentration in the study soil with those of other published study around the world. The polluted soil investigated in the current study can be considered to be within or much lower PAHs (Table 4-9 and Figure 4-28) if compared to those reported for soil samples from other parts of the world as shown in Table 4-10.

The PAHs results show that the concentrations of PAHs in soil samples vary. As the lower molecular weight PAHs are more volatile and soluble than the higher molecular weight PAHs, volatilization and leaching of the lower molecular weight PAHs might be responsible for some of the decrease in total PAH concentration observed in crude oil contaminated soils. Processes such as biodegradation will attack PAHs in sediments, leaving behind those PAHs that are resistant to degradation. Again it is mainly the high molecular mass PAHs that resist such attacks (Kanaly et al., 2000 and Bojes et al., 2007). It can be said that, soil samples were contaminated with PAH. The selected ions chromatograms of extracted PAH from soil collected from the study site are shown in Appendix E1 to E7. It can be seen from chromatogram the individual PAH peak of interest.

Table 4-10 PAHs levels in oil contaminated soil from oil and gas exploration and production site around the world ([a] Mean values)

Origin	No. of PAHs	ΣPAHs (mg/kg)	Sample source	Reference
Libya, present study	15	11.73-40.94	Oilfield	Present study
China, 2010	16	1.340-82.39	Oilfield soil	Lang et al., 2010
Romania, 2009	16	38.52	Industrial area	Ene et al, 2012
Egypt, 2008	16	2854.6	Oil polluted desert soil	Diab[2] 2008
India, 2008	16	60.36	Oil refinery	Tiwari et al 2011
Texas, USA, 2007	16	31.0 -86.4	oil exploration and production sites	Bojes et al, 2007
North central Mexico, 2005	12	35-101	Oil storage and distribution station	Iturbe et al, 2005
France, 2004	16	839.7-1098.9	Industrial area	Potin et al, 2004
Niger Delta, Nigeria, 2003	16	384-433[a]	Oil refinery and loading point	Abbas et al, 2005
Novi Sad (Serbia and Montenegro), 2002	16	47.90[a]	Industrial area	Skrbic et al 2002
Alberta, Canada, 1997	16	21.6-236.5	Oilfield soil	Zemanek et al, 1997
El Dorado, AR, USA, 1997	7	26.47	oil storage/separation facility	White et al, 2006
Australia, 1994	18	0.3–79	Industrial area	Sojinu et al., 2010
Tunisia, 2011	17	11-1124	Oilfield soil (evaporation pit)	Aloulou et al., 2011

4.4 Conclusions

Identification of pollutants allows for development of reliable treatment design criteria to help ensure that effective and consistent treatment is achieved to meet guideline levels required for disposal, or other beneficial uses. This chapter describes a study of analytical technique including IR, GC–FID, GC–MS and SPME techniques used to identify and characterize petroleum hydrocarbons in soil and produced water (PW) samples. Our results and data interpretation clearly indicate that:-

- PW samples were contaminated by TPH, PAHs and BTEX compounds and when produced water is released onto the open environment these contaminants will cause serious damage to the ecosystem.
- The results indicated that highly contaminated PW at the Nasser oilfield discharged directly without any sort of treatment into the surface soil onto open environment and this is of environmental concern.
- The PW is the main source of pollution; many constituents are carried by it, especially; hydrocarbons, disposed directly to the environment, it causes environmental impact.
- The results obtained showed that a substational amount of crude oil is being discharged with the PW, so that pollution prevention opportunities are going to be most effective when they are coordinated with the production sector, where efficiency of separation could be increased by developing of separation performance and technique, in order to prohibit or at least decrease oil free phase discharging with PW.
- Results from this study indicate that SPME coupled to GC–FID is a precise method for reproducibly analyzing BTEX inPW.
- Total Petroleum Hydrocarbon (TPH) and polyaromatic hydrocarbons (PAHs), concentrations of soil samples taken from the area around the Nasser oilfield disposal pit were quantified and the results showed elevated pollution with TPH and PAHs. This is real and evident that the detected TPH and PAHs in soil samples were shown to have originated from oily PW disposal which considered the main source of the contamination at the study site.
- A soil sample was definitely affected by the disposal of PW.
- It was observed that the soil was suffering from high pollution by hydrocarbons and this is an expected result due to continuous accumulation of crude oil carried by produced water.

Chapter Five
Analysis of oilfield chemicals in soil and produced water using ESI/MS/MS and LC/ESI/MS techniques

5.0 Introduction

As detailed in the introduction, produced water (PW) is by far the largest contaminated stream resulting from oil and gas recovery operations. Contaminants in PWs are toxic and corrosive leading to environmental and operational problems (Deriszadeh et al., 2010 and Ebrahimi et al., 2012). A number of oilfield chemicals (OFCs) are used during crude oil and natural gas recovery, production and separation to overcome operational problems and play a very important role in oil production (Grigson et al., 2000). These chemicals such as scale inhibitors, corrosion inhibitors, biocides inhibitors and demulsifiers are usually added to the top processing equipment of the oilfield to assist separation of produced fluid [oil, gas and water] and to protect the facility. OFCs are used for various purposes in oil production and these include scale inhibitors to avoid mineral scale deposition blocking pipelines, corrosion inhibitors to protect production equipment from corrosion attack, biocides to prevent bacterial degradation of oil and other products, and demulsifiers to help in oil water separation (Henderson el al., 1999 and Grigson et al., 2000). When PW is separated from crude oil and discharged into the disposal pit, some or all of these oilfield chemicals may be present in it. These may significantly affect the environment (Henderson el al., 1999 and McCormack et al., 2001).

According to Hudgins (1994) and McCormack (2001) an estimated 84097 tones of drilling chemicals and about 5934 tones of OFCs were discharged along with PW into the North Sea in 1989 (Hudgins 1994 and McCormack et al., 2001).

OFCs are generally complex formulations of compounds manufactured from impure raw materials made from natural products. Many of the active components are of high molecular weight. They are structurally complex and are sometimes charged compounds (Grigson et al., 2000 and Gagliardi et al., 2003). The complex nature of these formulations can make rigorous determination of solubility, composition, distribution coefficient, etc., virtually impossible in a classical sense. Some or all of these chemicals may be discharged to the environment along with the PW (Hudgins 1994, Henderson et al., 1999 and Stephens et al., 2000).

In general OFCs are often long chain fatty acids derived from natural products. Materials such as coco oil (C_{12}-C_{16}), tall oil (C_{18}-C_{20}) etc are often used as starting material for synthesising actives such as imidazolines (Grigson et al., 2000; Gagliardi et al., 2003). This results in actives containing a range of molecular weights reflecting, primarily, the alkyl chain lengths and degree of saturation of the raw materials. Quaternary ammonium compound formulations derived from tertiary coco amines

which have been quaternerised with a reactive alkylation reagent such as benzyl chloride or dimethyl sulphate. Fatty and ether amines with alkyl chain lengths ranging from C_{10}-C_{18} may be produce a range of ethoxylated fatty wthe it is react with ethylene oxide producing a range of different properties (i.e increasing water solubility with addition of ethylene oxide) (Gagliardi et al., 2003).

5.1 Types of production chemicals

5.1.1 Corrosion inhibitors

Corrosion is one of the major serious problems in crude oil and gas production facilities which are subject to corrosive attack by gases such as carbon dioxide (CO_2), hydrogen sulfide (H_2S) and other constituents that may be present in the reservoir fluids (oil. gas and water). Inhibitors are chemicals that react with the surface of metal and which are supposed to give the surface protection. These often work by adsorbing and protecting the metallic surface by forming a film. A corrosion inhibitor is a chemical compound that when added in small concentrations will stop or slow down corrosion in a material (McMahon 1991).

Corrosion inhibitors can either be oil-soluble/water dispersible or water-soluble/oil dispersible. The type of corrosion inhibitors depends upon whether the metal or alloy to be protected is water-wet or oil- wet, while in general oil-soluble inhibitors are preferred for oil production because of their greater effectiveness. All the corrosion inhibitors used are organic compounds that form a protective layer on the target metal surface (Henderson 1999 and Gagliardi et al., 2003). Most corrosion inhibitors contain nitrogen as the functional group. Nitrogen containing material will be reacted with a carboxylic acid to form a compound that can be optimized for a variety of different applications by altering the reaction conditions (Henderson 1999 and Gagliardi et al., 2003). Corrosion inhibitors can commonly be classified by the chemical structure of their active component(s):

* Amide/imidazoline: Most corrosion inhibitor formulations have one or both compounds present. Amide/imidazoline are complex mixtures and usually have high molecular weight and low solubility in water.

* Amine/amine Salts: Salt mixture contains mostly long chain monoamines (C_{10}-C_{18}); the water solubility depends upon the length of and degree of ionisation of the carbon tail.

* Nitrogen Heterocyclic: are organic compounds with functional groups of aromatic or aliphatic ring structure containing a nitrogen atom, usually they have little or no water solubility.

* Quaternary Ammonium Salts: (also known as quats), are quaternary ammonium-based surfactants and are mainly produced from natural fats and oils resulting in mixed alkyl chain lengths in some products, (Garcia et al., 2001 and Gagliardi et al., 2003). Quaternary ammonium compounds (QACs) are a nitrogen compounds in which a central nitrogen atom is joined to four organic radicals and one acid radical (R4N+). Functional groups (R) include at least one long chain alkyl group and the rest are either methyl or benzyl groups. QACs have been used in large amounts for more than 30 years ago (Garcia et al., 2000 and Kaech et al., 2001). The active component in QACs may be a molecule like trimethylalkylammonium chloride, with an alkyl side chain most likely comprising a complex mixture of long chain hydrocarbons, and all quaternary ammonium salts have high water solubility (Henderson 1999 and Gagliardi et al., 2003). QACs containing a long-chain alkyl group and/or a benzyl group, are cationic surfactants and the active ingredient in many corrosion inhibitor formulations (Gagliardi et al., 2003 and Patrauchan et al., 2003).

5.1.2 Demulsifiers inhibitors

Demulsifiers are commonly used in the production of oil. As crude oil is produced from a reservoir it tends to become mixed with gas and natural FW. This water can significantly change the properties of oil. It is important, however, for the majority of that water to be separated from the mixture before the crude oil is refined or exported (Deriszadeh et. al., 2010). The gas is separated first at one or two pressure levels, and this is followed by the separation of PW. Due to large volume proportions of PW present at the oilfield some will be in the form of free water which is easy to separate from the oil. Some water will however be present as an emulsion (i.e. water droplets) (Hudgins 1994). It is critical to topside process operations that the oil is efficiently and quickly separated from the water to allow dry oil to be exported and PW to be discharged (Deriszadeh et. al., 2010). Demulsifiers are blends of surface active agents, normally supplied in a solvent. Anionic, cationic and non-ionic demulsifiers can be used, depending on the specific nature of the emulsion. The most common compounds are oxyalkylated alkyl phenol formaldehyde resins, polyglycol esters, and alkyl and aryl sulphonates. Almost all formulations contain more than one of these generic types as well as surfactants. The components of these formulations tend to be very insoluble in water and distribute into the oil phase (Hudgins 1994 and Gagliardi et al., 2003).

5.1.3 Scale inhibitors

Scale inhibitors are a chemical treatment used to control or to prevent scale deposition in the production equipment or completion system. A thermodynamic chemical

equilibrium is often created between dissolved solids in PW and downhole conditions. As the temperature and pressure of fluids (oil, gas and water) become lowered once they reach to the surface, the chemical equilibrium of PW will be changed. This change will cause the precipitation of inorganic salts in the production equipment. Scale deposition inside pipelines and equipment occurs often when the PW becomes supersaturated with the carbonate or sulfate salts of barium, calcium and strontium as well as changing in temperature and pressure of the fluids in well (Henderson 1999). Scale inhibitors must be added to the equipment and facilities at the oilfield to reduce mineral scale deposition, because scale inhibitors are soluble in water they are discharged to the open environment along with PW. The organic chemicals used in the scale inhibitors are polyphosphate esters of amino alcohols, phosphonates or acrylic acid type polymers (sodium polyacrylate polymers). These chemicals absorb into the crystal nuclei as scale first precipitates and prevent further deposition (McCormack et al., 2001).

5.1.4 Biocides

Biocides are used in the oil industry to prevent biofilm formation and to control anaerobic and aerobic bacteria (Kelland 2009). Biofilm is a major problem when dealing with the large quantities of produced water associated with crude oil production. The biofilm, itself a major problem encourages microbial growth which will produce hydrogen sulphide by biochemical reduction of sulphates. The presence of bacteria can impact production operations, leading to the occurrence of bacterial fouling of equipment and hydrocarbon degradation. Furthermore, iron sulphide and degraded oil will be produced at the oil/water interface in the tanks which will affect the efficiency of separation. To prevent corrosion or fouling of the equipment and facilities various bacteria, especially sulfate reducing bacteria, should be controlled. The most commonl chemicals used in biocides are aldehydes, quaternary ammonium salts, and amine acetate salts. All the biocides are highly water soluble, as they are designed to be effective in the water phase (Reis 1996 and Kelland 2009).

5.1.5 Paraffin treating chemicals

Crystallisation of aliphatic long chain hydrocarbons (wax) occurs in crude oil as the temperature decreases from the reservoir to the settled tank. In addition, this may cause plugging in the facilities and pipelines (Kelland 2009). The use of paraffin treating chemicals at the oilfield is to control the accumulation of solid hydrocarbons in the production equipments. Paraffin treating chemicals control the growth of paraffin crystals by reducing the tendency of the crude oil to deposit. The most commonly used compounds include vinyl polymers, sulphonate salts and mixtures of alkyl polyethers

and aryl polyethers. Paraffin solvents are used to remove accumulations of deposits. All these class of chemicals are more soluble in oil than in PW (Henderson 1999 and Kelland 2009).

5.1.6 Emulsion breakers and De-oilers

PWseparated from crude oil often contains dispersed oil droplets. It is necessary to reduce the residual amount of oil in PW prior to disposal or re-injection. Polyamine or polyamine quaternary ammonium compounds are added to convert small oil droplets into larger oil droplets (emulsions reverse breakers). These chemicals are highly water soluble, possibly PW will contain some of this chemical and will be discharged into environment along with it (Henderson 1999 and Kelland 2009).

The presence of these chemicals in PW has been shown to be toxic in standard toxicity test. Furthermore oilfield chemicals in PW can affect the oil/water partition coefficient, bioavailability, and biodegradability (Grigson et al., 2000 and Manfra et al., 2010). OFCs poses the most concern for water toxicity include biocides, reverse emulsion breakers, and corrosion inhibitors (Brendehaug et al., 1992 and Utvik 1999). However, these substances may undergo reactions that reduce their toxicities before they are discharged or re injected. For instance, biocides react chemically to lose their toxicity, and some corrosion inhibitors may partition into the oil phase so that they never reach the final discharge point (Grigson et al., 2000 and Veil et al., 2004).

Since several of the organic chemicals in the OFCs are polar, hydrophilic compounds, they are not suitable for analysis by techniques such as GC-MS. Additionally; many of the chemicals of importance are surface active making the development of assays for their analysis in environmental samples particularly difficult (Langley et al., 1999 and McCormack et al., 2001). Establishment of the methods is therefore very important to OFCs manufacturers, oil companies and to the scientists concerned about the fate effects of PW and OFCs in the environment (Washburn et al., 1999, McCormack et al., 2001 and Gagliardi et al., 2003).

5.2 Principle of ESI-MS technique

5.2.1 Mass spectrometry (MS)

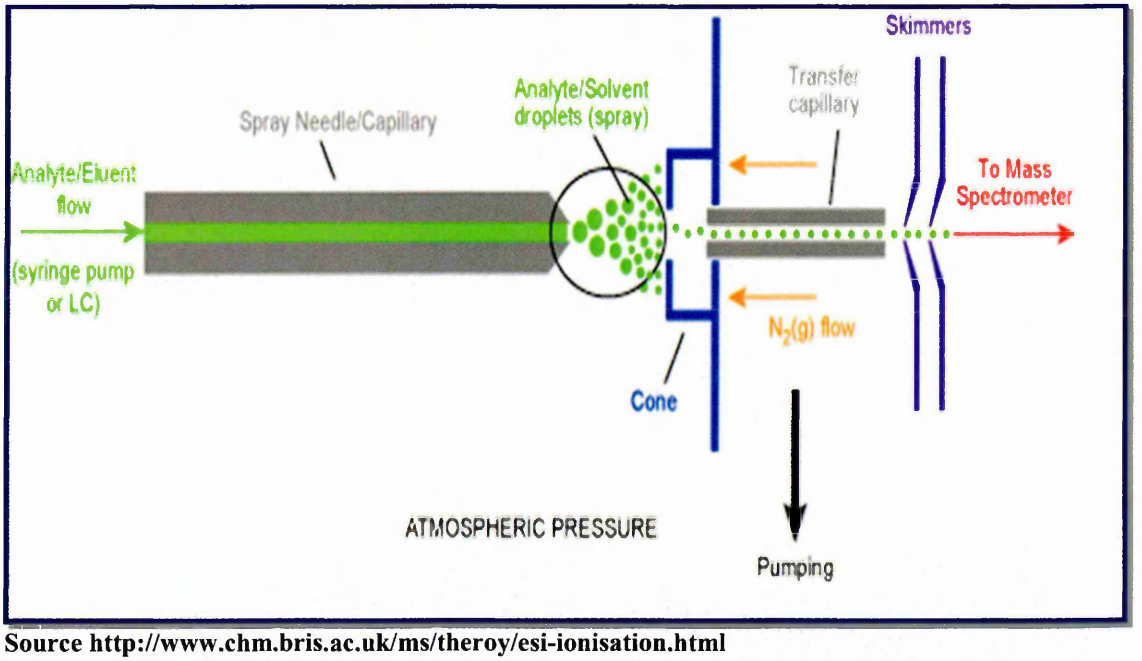
Mass spectrometry is an analytical tool used for measuring the molecular mass of a sample, which can give both quantitative and qualitative information on analyte molecules after their transfer to ions. Molecules are first introduced into the ionization source of the mass spectrometer, where they are ionized to acquire positive or negative charges (Langley et al., 1999, Ho et al., 2003 and Hoffmann et al., 2007). These ions are extracted into the anaylzer region of the mass spectrometer where they are separated

according to their mass/charge (m/z) ratio. After the ions make contact with the detector, signals are generated and sent to a computer system. The computer displays the signals graphically as a mass spectrum showing the relative abundance of the signals according to their m/z ratio (Hoffmann et al., 2007 and El-Aneed et al., 2009).

5.2.2 Electrospray ionisation (ESI)

The development of electrospray ionisation (ESI) started with the work of Dole and coworkers, who successfully introduced a polystyrene polymer into the gas phase as a charged species in 1960s (El-Aneed et al., 2009).

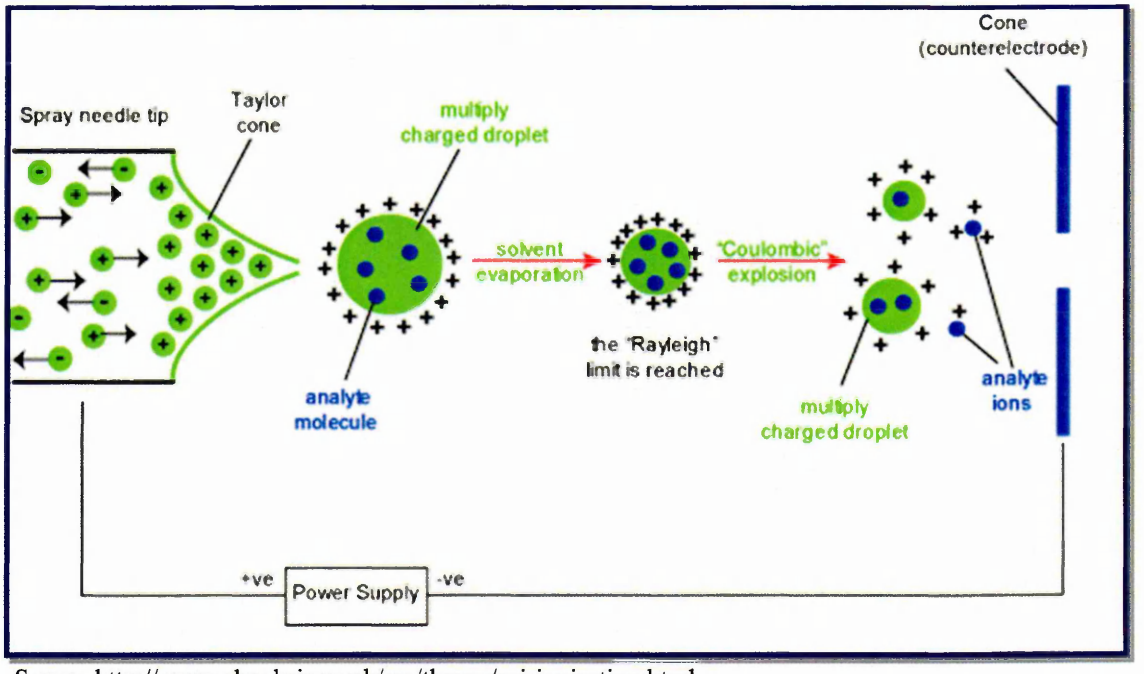
ESI is an atmospheric pressure ionisation technique in which ionisation is achieved by applying under a strong electrical field to assist the transfer of ions from solution into gaseous phase (Ho et al., 2003, Richard 2009 and El-Aneed et al., 2009). After ionisation takes place at atmospheric pressure ions are then transferred into the vacuum system of the MS. The analyte is introduced to the source in solution either from a syringe pump or as the eluent flow from liquid chromatography (Ho et al., 2003, Hoffmann et al., 2007 and Cole 2010). The analyte solution flow passes through the electrospray needle that has a high potential difference applied to it. This forces the spraying of charged droplets from the needle with a surface charge of the same polarity to the charge on the needle. The droplets are repelled from the needle towards the source sampling cone on the counter electrode. As the droplets traverse the space between the needle tip and the cone then the solvent evaporation occurs. Figure 5-1 shows a schematic of an electrospray ionisation (ESI) source.



Source http://www.chm.bris.ac.uk/ms/theroy/esi-ionisation.html

Figure 5-1 Schematic of an electrospray ionisation (ESI) source

These charged droplets shrink as neutral solvent evaporates until the charged density exceeds the Rayleigh limit and coulombic repulsion causes the droplet to divide as shown in Figure 5-2. In addition, highly charged droplets are produced under the strength of the electrical field being applied. When the electrical field on ions surface is large, the ions are desorbed large molecules with several ionisable sites produced multiply charged ions by ESI (Hoffmann et al., 2007 and Cole 2010).



Source http://www.chm.bris.ac.uk/ms/theroy/esi-ionisation.html

Figure 5-2 Schematic of the mechanism of ion formation in ESI

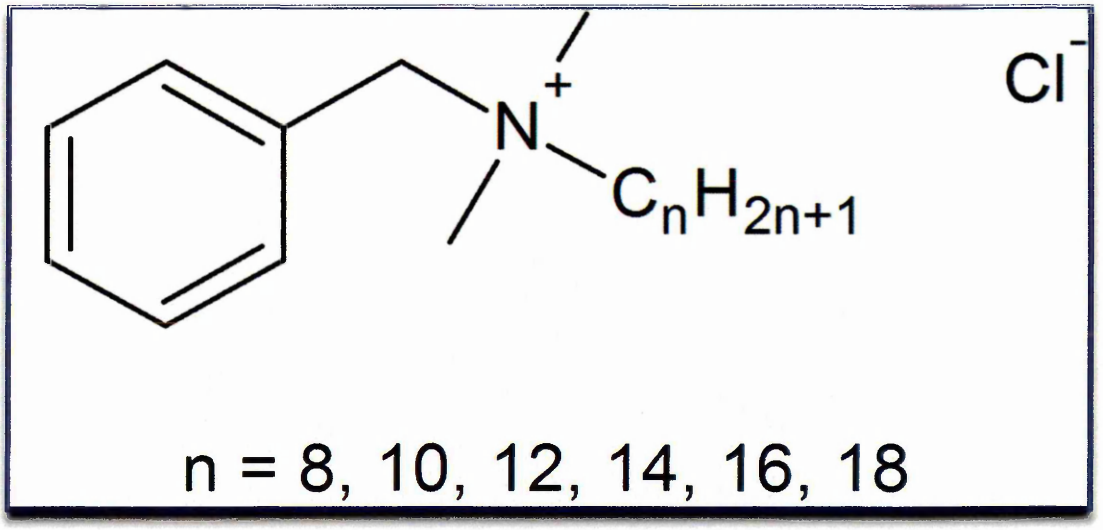
ESI-MS is one of the most common techniques used for quantitative and qualitative analysis of petroleum based chemicals, OFCs and other polar chemicals in environmental and industrial sample (McCormack et al., 2002 and Porter et al., 2004).

ESI-MS is an important technique in environmental analysis as it provides a sensitive, robust, and reliable tool for studying unknown compounds in samples (Ho et al., 2003), ESI can produce abundant ions from both basic and acidic nitrogen heterocycles in positive and the negative ionisation mode, respectively (Panda 2007). In additional using the separation capabilities of MS in a series, commonly denoted as (MS/MS), complicated sample analysis can be much simplified (Ho et al., 2003).

The introduction of the ESI-MS interface, and subsequent developments in the methodology that occurred in the mid-1990s, have now provided the potential to study in detail the chemistry of OFCs (Grigson et al., 2000). Typical chemicals found in the oilfield i.e. corrosion inhibitors, scale inhibitors and demulsifies had been analyzed using ESI-MS, including some investigations of real polluted soil and PW samples (Langley et al., 1999).

There are major problems in measuring oilfield chemicals by ESI-MS mainly in the mass spectrometer measurements caused by the high salt content and wide range of organic compounds present in PW. These may interfere with the analysis of the oilfield chemical of interest. Therefore solid phase extraction (SPE) has been used to remove the salt and extract the OFCs of interested to be analysed (Gagliardi et al., 2003).

This Chapter describes the investigation and summarizes experiments and results obtained from the analysis of OFCs specifically corrosion inhibitors and demulsifiers in soil and PW by the use of ESI-MS/MS and LC-ESI-MS. This study focussed on benzalkonium (QUATs) or alkyl dimethyl benzyl-ammonium salts, where the alkyl side chain is a long hydrocarbon chain, typically from C_8-C_{18} as shown in Figure 5-3. A qualitative characterization of this main group of oilfield chemicals in soil and PW of samples collected from the Nasser oilfield, Libya, has been carried out. In addition, this chapter presents a review of the nature and function of commercial oilfield chemicals that are commonly used in the Nasser oilfield.



Source: Sigma Aldrich

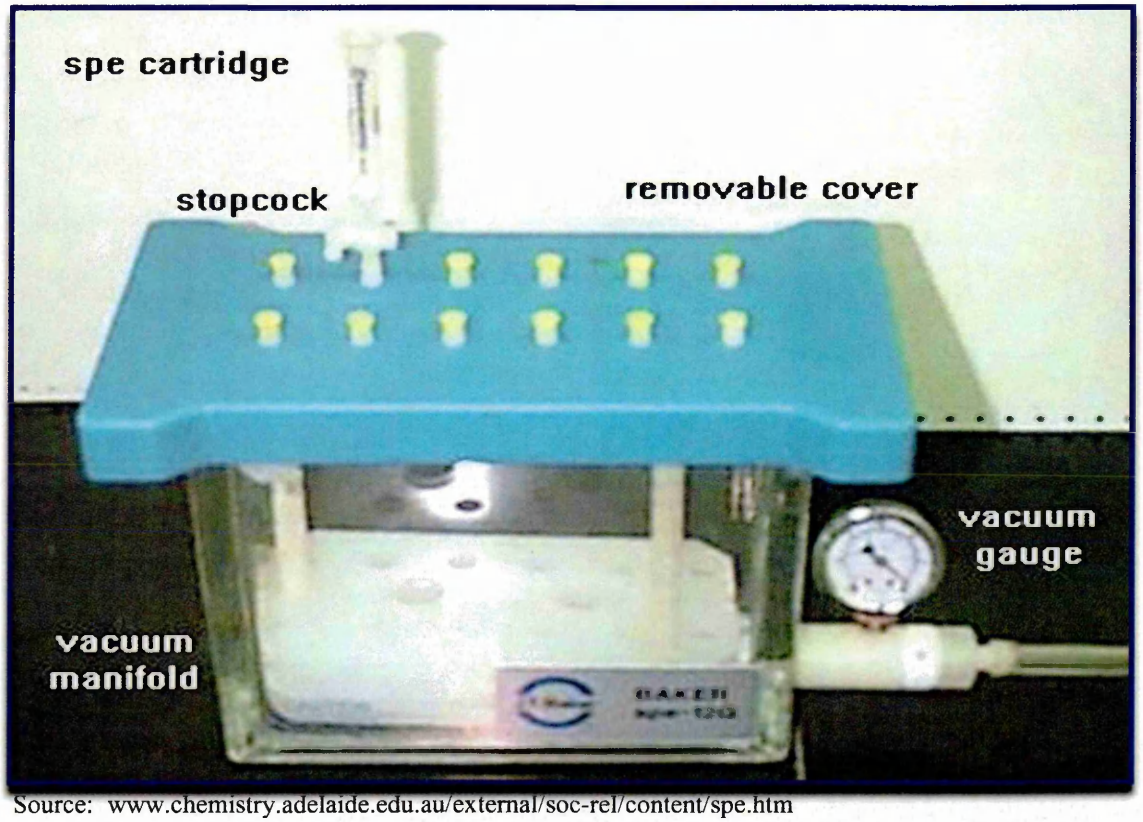
Figure 5-3 Structure of the quaternary ammonium salts (QUATs) or benzalkonium chloride

5.2.3 Solid phase extraction (SPE)

SPE is very useful extraction technique that is used for rapid and selective sample preparation and purification of complex samples to isolate one type, of analyte from a solution and / or concentrate low levels of analytes, prior to chromatographic analysis, since it is rapid, and has low operating costs and environmental impact (Gagliardi et al., 2003, Zwir-Ferenc et al., 2006 and Faghihian et al., 2010). SPE is usually used to clean up a sample before using a chromatographic or other analytical method to determine the amount of analyte(s) in the sample. Furthermore, SPE is employed to prepare samples for subsequent analysis by removing interfering substances that may be present in the

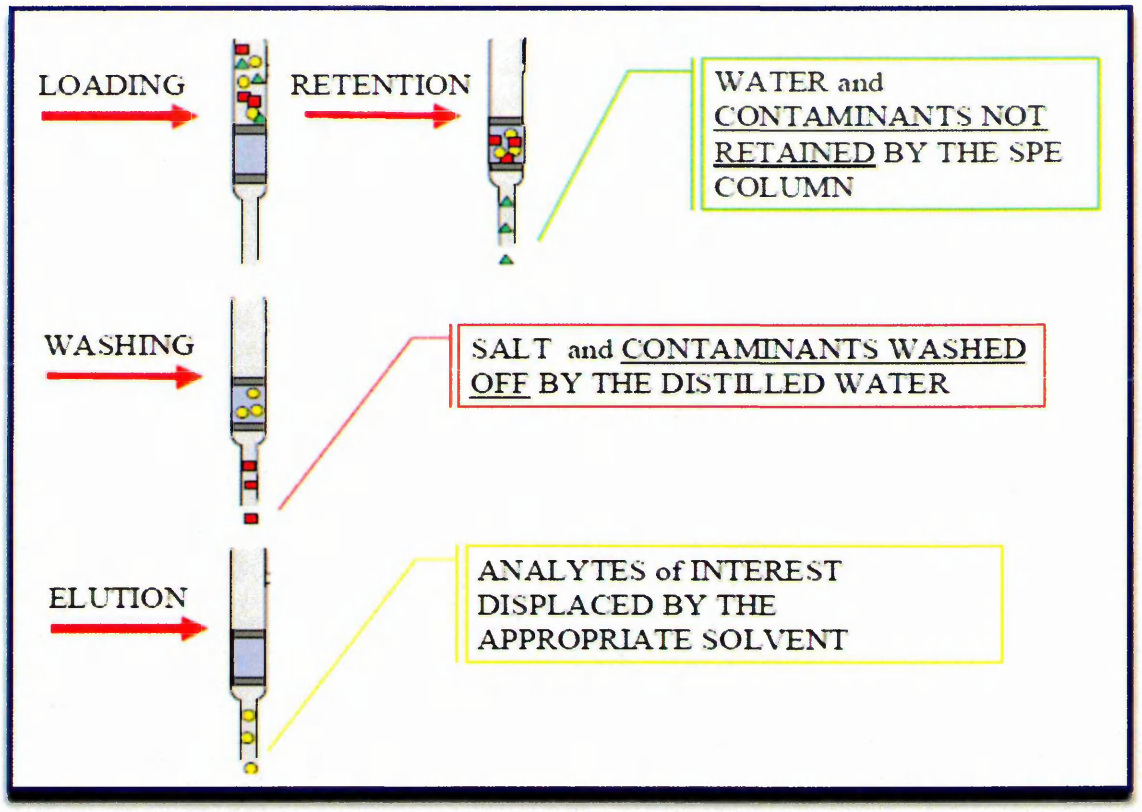
sample by washing off with a suitable solvent and eluting the compound of interest by another solvent (Zwir-Ferenc et al., 2006). The SPE apparatus consists of simple cartridges have 1-10 ml capacities which are usually packed with an appropriate bonded phase and are discarded after use. The vacuum manifold, which increases the solvent flow rate through the cartridge, plus a collection tube which is placed inside the vacuum manifold and beneath the SPE cartridge to collect the compound of interest from the liquid that passes through the column as shows in Figure 5-4 (Zwir-Ferenc et al., 2006). There are many different types of adsorbents that have been used for solid phase extraction such as thiol cotton activated carbon, cellulose, solvent-impregnated resins, microcrystalline naphthalene, Amberlite XAD-2 resin, alumina, silica, agar, clays and zeolite (Faghihian et al., 2010).

In general the procedure used is to load a sample onto the SPE tube, wash away undesired components, and then the compound of the interest is eluted with another solvent into a collection tube (Zwir-Ferenc et al., 2006). Figure 5-5 shows the SPE clean up steps. Solid phase extraction procedures are used not only to extract traces of organic compounds from environmental samples but also to remove the interfering components of the complex matrices in order to obtain a cleaner extract containing the analytes of interest. SPE extends a chromatographic systems lifetime and improves qualitative and quantitative analysis (Langley et al., 1999).



Source: www.chemistry.adelaide.edu.au/external/soc-rel/content/spe.htm

Figure 5-4 Illustration of a solid-phase extraction set-up



Source: www.og.decc.gov.uk/environment/Charmval Project (HeriotWattUni).pdf

Figure 5-5 General principles of reversed phase extraction (SPE) cartridge

5.3 Materials and methods

5.3.1 Sampling

The soil and PW samples were collected from the Nasser Oilfield as described in Chapter two sections 2-11-6. In summary the PW came from 4 sampling locations, and 18 soil samples were collected from different locations around the discharge pit as shown in Figures 2-17 and 2-18. Seven commercial oilfield chemicals used at the Nasser oilfield (corrosion inhibitors, scale inhibitors, biocide and demulsifier) were also obtained. The ESI-MS-MS and reversed-phase HPLC separations LC coupled with ESI-MS were used to analyse the OFCs.

5.3.2 Chemicals and suppliers

Dichloromethane (DCM) and methanol used were of HPLC grade and were obtained from Sigma-Aldrich, UK. Sodium sulphate was of AR grade and purchased from Fisher Scientific (UK). All water used was Milli-Q grade and C18 SPE cartridges (500mg/3ml) were purchased from Supelco, UK. Dodecylbenzenesulfonic acid (DBSA) sodium salt and benzyl-dimethyl-tetradecyl-ammonium chloride (BDMTDA) were obtained from (Sigma-Aldrich, UK). Commercial OFCs samples used in this study were obtained from the Nasser Oilfield, Libya.

5.3.3 Proprietary of commercial oilfield chemicals

Seven proprietary commercial oilfield chemicals (four corrosion inhibitors, two scale inhibitors, one biocide and one demulsifier) which are used to protect production equipment and facility from corrosion and scale deposition and to help oil water separation were chosen for this study. The material safety data sheets (MSDS) for these chemicals as used in the Nasser Oilfield, Libya were studied to see what information could be gained. The description and some information taken from MSDS for each is as follows:-

5.3.3.1 Corrosion inhibitor EC1180A:- is a commercial corrosion inhibitor from the Ondeo Nalco Ltd. Company used in the study area. It is stated to contain (MSDS) 1-benzyl-1-(2-hydroxyethyl)-2 coconut oil-2- imidazolinium chloride (10.0–30.0 %), benzyl C10-16 alkyldimethyl ammonium chloride (5.0-10.0 %), ethylene glycol (5.0-10.0 %) and isobutanol (1.0-5.0 %). It is a liquid with an alcoholic odour and is completely soluble in water.

5.3.3.2 Corrosion inhibitor CHIMEC 1053:- is a balanced mixture of compounds with a different volatility providing an appropriate film on all metal surfaces it is recommended as corrosion inhibitor for equipment of oil and gas production in oilfield from CHIME Company used in the study area. It is stated to contain a blend of aliphatic amines and amine derivates in a high boiling point solvent (1, 2, 4 trimethylbenzene). It is a brown liquid and is completely soluble in aromatic hydrocarbons.

5.3.3.3 Corrosion inhibitor CRW2772:- is a commercial corrosion inhibitor from the Baker Petrolite Company used in the study area to protect the facility at field from corrosion. It is stated to contain (MSDS) amide/imidazolines (10.0-30.0 %), quaternary ammonium salts (5.0-10.0 %), propan-2-ol (10.0-30.0 %), methanol (1.0-5.0 %), and acetic acid (1.0-5.0 %). It is a liquid with a characteristic odour and is soluble in water.

5.3.3.4 Corrosion - Biocide inhibitors EC6206A:- is a commercial biocide inhibitor from Ondeo Nalco Ltd. Company which is used in the study area. It is stated to contain (MSDS) 1-benzyl-1-(2-hydroxyethyl)-2 coconut oil-2- imidazolinium chloride (30.0–60.0 %), benzyl C10-16 alkyldimethyl ammonium chloride (5.0-10.0 %), ethylene glycol (1.0-5.0 %) and isobutanol (1.0-5.0 %). It is claimed to provide an excellent biocide effect particularly on sulphate reducing bacteria (SRB). It is a liquid with a mild odour and is completely soluble in water.

5.3.3.5 Demulsifier D6261:- is a commercial emulsion breaker from JOF BRAIK Company, Libya. It is blend of alkoxylate resins and high molecular weight polyols in an aromatic solvent. It is formulated to give good demulsification and water separation

in a range of medium to high API gravity crude oils. It is stated to contain, aromatic solvent (50–75 %), kerosene (5.0-15.0 %), block polymer (5.0-10.0 %), amine oxalkyate (5.0-10.0 %) and cationic surfactant (5.0-10.0 %). It is claimed to provide an excellent biocide effect particularly on SRB. It is a clear, bright, and yellow to light brown liquid with aromatic odour and is freely soluble with xylene and a white emulsion with water.

5.3.3.6 Scale inhibitor SCW2510:- is an excellent inhibitor for the control of carbonate scales and is also effective against sulphate scales. It is a blend of a phosphonate and a neutralised polyacrylate type scale inhibitor (MSDS) from Baker Petrolite Company which is used at the study area to protect the facility at the oilfield. It is a liquid with a characteristic odour and is exhibits good tolerance to medium to low TDS brines, and is completely soluble in most water.

5.3.3.7 Scale inhibitor EC6165:- is designed to prevent calcium carbonate, calcium sulphate and barium sulphate scales at very low chemical dosages in producing wells, water injection system and salt water disposal systems.. It comprises organic acid derivatives in water - alcohol solution and is a common type of scale inhibitor. It is stated to contain ethylene glycol (5.0-10.0 wt. %), alkanolamine phosphate (10.0-20.0 wt. %) and phosphoric acid (10.0-20.0 wt. %). It is a liquid with a slight odour and is water soluble and expected to remain primarily in water.

5.3.3.8 Benzyldimethyltetradecylammonium chloride (BDMTDA), is also known as benzalkonium chloride, is a mixture of alkylbenzyldimethylammonium chlorides of various even-numbered alkyl chain lengths, [i.e n-Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}), dimethyl benzyl ammonium chloride, and n-alkyl 68% C_{12} and 32% C_{14} dimethyl ethyl benzyl ammonium chloride] and the Chemical Classification is quaternary ammonium compounds.

5.3.4 Sample preparation

5.3.4.1 Preparation of standard

- Stock solutions of commercial oilfield chemicals (EC1180A, CHIMEC 1053, CRW2772, EC6206A, D6261, SCW2510 and EC6165) used at the Nasser Oilfield were prepared as external standard solution. The concentration was made up to 50 ppm in methanol and dilution of the stock was made using the required solvent prior to use of the ESI-MS-MS.
- Benzyldimethyltetradecylammonium chloride (BDMTDA) was typically made up to 50 ppm in methanol and dilution of the stock was made using the required solvent prior to use of the ESI-MS.

5.3.4.2 Soil samples preparation

100 gram of homogenized polluted soil sample was freeze dried and extracted with 3×50 ml MeOH in an ultrasonic bath for 20 min. The extract (about 30 ml) was put onto a 6-ml Discovery DSC-18 solid phase extraction (SPE) column (SPE cleanup column was first pre-conditioned with 5 ml MeOH followed by 5 ml ultra pure water) (Grigson et al, 2000), then the salt was removed from the extract by washing the column with 20 ml ultra pure water then 1 ml of acidified MeOH (0.1% formic acid) was added to the column to condition it. Finally the column with the isolated compounds was eluted with 6 ml dichloromethane (DCM) into a 10 ml volumetric flask. The DCM was evaporated under a gentle stream of nitrogen and the residue was re-dissolved in 90:10 (v/v) MeOH / water. Then the extract was analyzed for OFCs residues by ESI-MS and LC-ESI-MS (Grigson et al., 2000).

5.3.4.3 Produced water samples preparation

One litre of PW was extracted three times with 30 ml DCM. The extract was dried under nitrogen and the residue was re-dissolved in pure MeOH. A 30 ml portion of the extract was subjected to SPE procedures and flushed it from the column with ultra pure water to remove salt from the sample by, followed by the addition of 1 ml acidified MeOH (0.1% formic acid) to the column to condition it (Langley et al., 1999). Finally the column with isolated compounds was eluted with 6 ml DCM into a 10 ml volumetric flask. The DCM was evaporated under a gentle stream of nitrogen and the residue was re-dissolved in 90:10 (v/v) MeOH/water. Then the extract was analyzed for oilfield chemicals residues by ESI-MS and LC-ESI-MS (Grigson et al., 2000).

5.3.5 Instrumentation

5.3.5.1 Electrospray ionisation tandem mass spectrometer (ESI-MS)

In this investigation isolated chemicals of interest were analysed using a Finnigan Mat LCQ (San Jose, CA, USA.) quadrupole ion trap. The samples were injected into an ESI mass spectrometry source using a high pressure liquid chromatography syringe. The operating conditions were injector loop: 250 µl; flow rate: 3 µl/min; carrier solvent was a mixture of acidified MeOH and water 90:10 (v/v); Capillary voltage: 13 KV; Spray voltage 4.5 KV Capillary temperatures 150°C, the data were processed using the Xcalibur software provided by Thermo Finnigan.

5.3.5.2 Liquid chromatography electrospray ionisation mass spectrometer (LC/ESI-MS)

Reversed- phase HPLC separations were performed using LC equipped with a pumping system (JASCO PU 980 HPLC Pump), detector and coupled to the ESI-MS (Finnigan

Mat LCQ, San Jose, CA, USA). LC separation was conducted on reverse phase column type C18, Phenomenex 150 x 1.0 mm. The eluent was a mixture of acidified MeOH and water [90: 10 (v/v) MeOH: water]. The operating condition were the capillary voltage was set at 13 KV, the spray voltage at 4.5 KV and the tube lens offset at 15 KV. The samples were analyzed by LC-ESI-MS in positive ion mode and the mass spectra were acquired and processed using the Xcalibur software provided by Thermo Finnigan.

OFCs of interest were qualitatively analyzed in soil and PW by ESI-MS-MS and LC-ESI-/MS/. Significant ions in full scan spectra [m/z 50–1850]; were acquired in order to provide data that could be used to identify the unknown extracted compounds (Langley et al., 1999 and Gagliardi et al., 2003). This was done by using stock solutions of commercial production chemicals used at the Nasser oilfield as external standards to identify the series of benzalkonium compounds of the ion peaks m/z 304.4, 332.2, 360.2 and 388.1 on ESI-MS/MS. These represent the molecular ions of C_{12}, C_{14}, C_{16} and C_{18}. These were completed by integrating and determining the m/z and relative abundance of analyte and compared with the m/z and relative abundance of external standard peaks.

5.4 Results and discussions

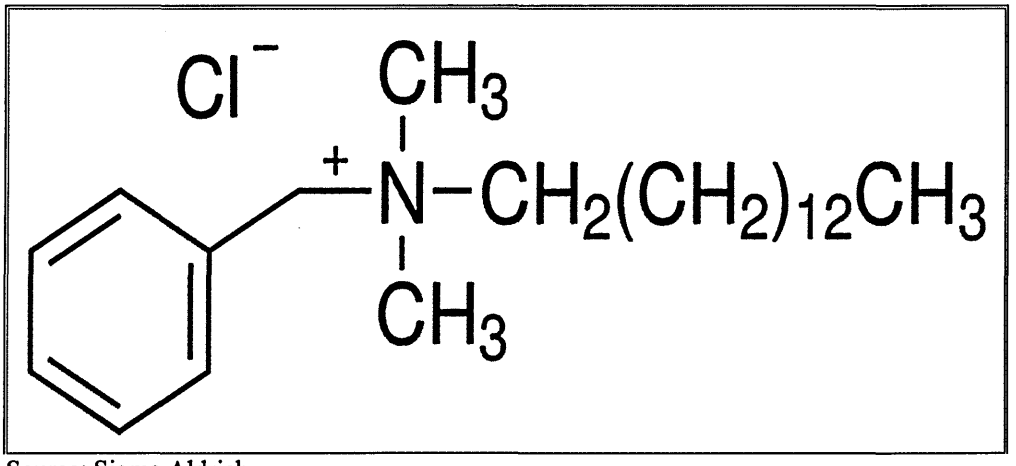
As mentioned above, PW is a complex mixture of inorganic and organic compounds. PW discharged into the open environment contains production chemicals (i.e. OFCs) which are used by the oil company to help oil water separation and to protect production equipment from corrosion and scale formation. Scale formation and corrosion are a serious problem encountered in many industries including oil and gas production (El-Said et al., 2009). A large range of OFCs (corrosion inhibitors, scale inhibitors and demulsifiers) are used in the Nasser Oilfield. The information given regarding the active chemicals is commonly limited to the classification of compounds (e.g. amines, quaternary amines, imidazolines, and phosphonates) and the solvents used i.e. aqueous, methanol or aromatic. Such chemicals can be released into the environment associated with PW. Due to the toxicity of some of these chemicals there is concern over the impact they may have on the ecosystem. In this study, different OFCs used at the Nasser Oilfield were studied by ESI/MS/MS and LC//ESI/MS/MS. Soil and PW samples collected from the study site were also analyzed for the presence of OFCs. The presence of interferences from the sample matrix, made the use of MS-MS necessary for the measurement of the OFCs. However, the use of liquid chromatography coupled with electrospray ionisation mass spectrometry LC//ESI/MS/MS showed that it is useful for the detection of OFCs, in soil and PWs. In this study different techniques were used to analyses soil and PW samples containing oilfield chemicals (OFCs): DCM extraction and SPE. These initial experiments into the use of ESI/MS/MS and LC/MS/MS have shown that it is useful for the detection of OFCs, in soil and PW.

5.4.1 Qualitative analysis by ESI/MS technique

5.4.1.1 Analysis of pure chemicals and commercial oilfield chemicals

Many commercial OFCs are blends of two or more chemical types, the specific chemicals and quantities contained in oilfield products are not generally made public and only the legally required health and safety data are normally specified on material safety data sheet. Without detailed knowledge of the oilfield chemicals compositions it is difficult to identify and measure these unknown chemicals in the environment. A preliminary analysis was carried out in which samples of commercial OFCs used at the Nasser oilfield were prepared in methanol: water (9:1 v/v) and injected into the ESI-MS in the positive ion mode (m/z 50–1850) prodction mass spectra were been obtained for significant ions in these samples in order to provide data that could be used to identify the compounds in the produced water samples and the results are as follows:

Benzyldimethyltetradecylammonium chloride (BDMTDA), a pure standard chemicals (also known as benzalkonium chloride), is one example of a quaternary ammonium salt represented by the structural formula as shown in Figure 5-6. It is a mixture of alkylbenzyldimethylammonium chlorides contain 60% of C_{14}, 30% of C_{16}, 5% of C_{12}, 5% of C_{18}), and is a quaternary ammonium compound. Positive ion ES-MS full scan mass spectrum (m/z 50–1850) of pure BDMTDA dissolved in acidified methanol: water (90: 10, v/v) was obtained along with product ion spectra of significant peaks were acquired in order to provide data that could be used to identify the unknown extracted compounds (Figure 5-7). The chromatograms of BDMTDA (Figure 5-7), indicate that the main ion peak in this standard was m/z 332, which would correspond to the structure of the C_{14} homolog. This is expected result as these standard according to MSDS, started to contain 60% C_{14} in it is structure. The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 were subject to CID to give products ions of m/z 212, m/z 240 and m/z 267 respectively as shown in Figure 5-8. These represent the molecular ions of C_{12}, C_{14} and C_{16} which are the series of benzalkonium quaternary ammonium salts.



Source: Sigma Aldrich

Figure 5.6 Benzyldimethyltetradecylammonium chloride [BDMTDA] Structure

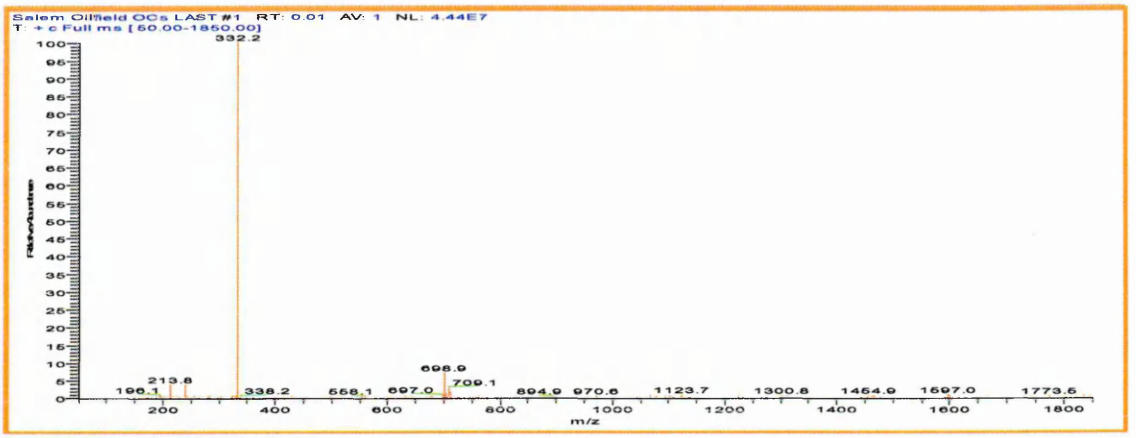


Figure 5-7 ESI mass spectra full scan (m/z 50-1850) of benzyldimethyltetradecyl ammonium chloride (BDMTDA)

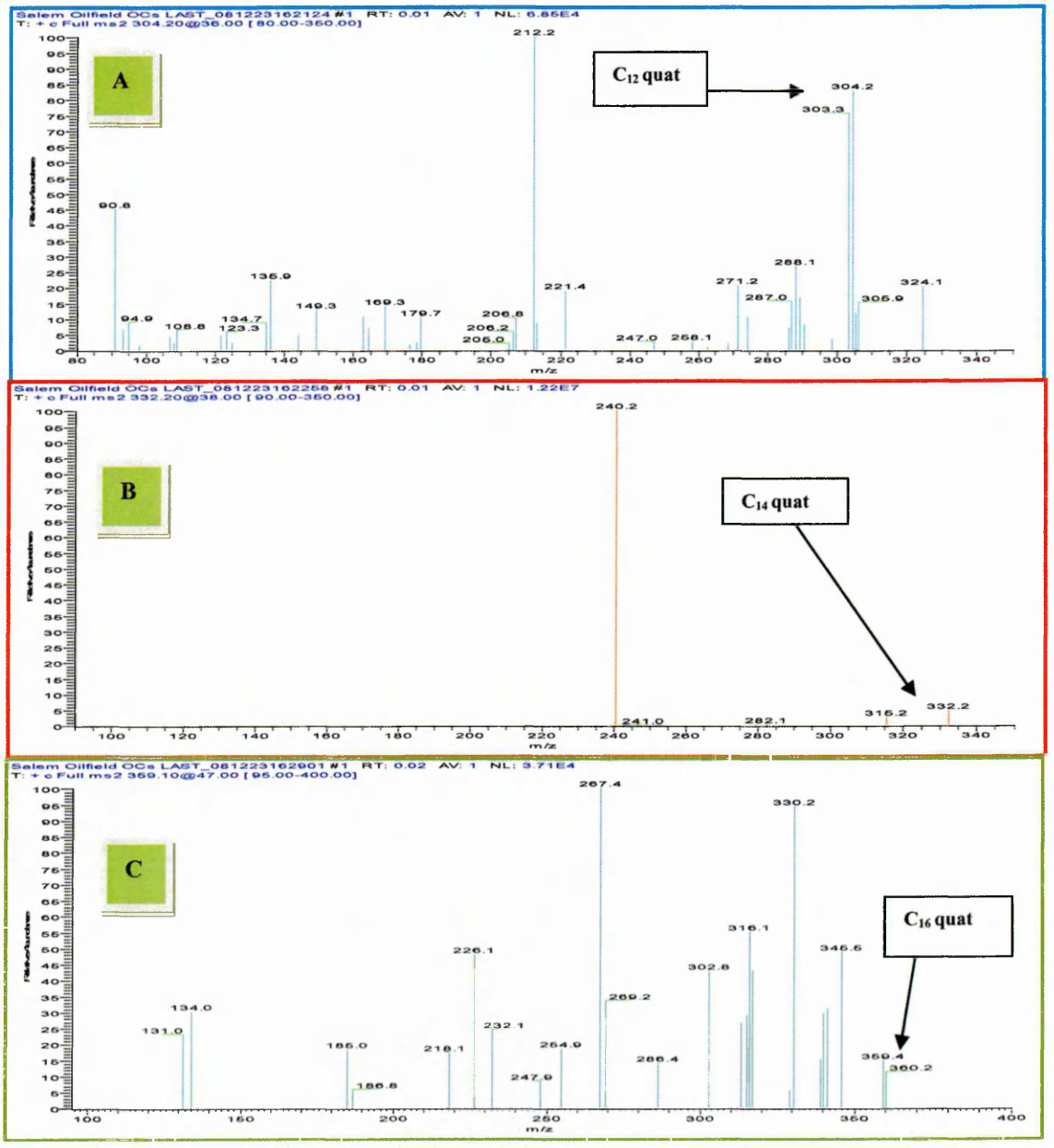


Figure 5-8 ESI-MS^2 mass spectra of ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 360) of pure standard BDMTDA

Corrosion inhibitor CRW2772 is a commercial oilfield corrosion inhibitor used at the Nasser Oilfield and stated to contain Amide/Imidazolines and quaternary ammonium salts according to its material safety data sheet (MSDS). Positive ion ES-MS full scan mass spectrum (m/z 50–1850) (Figure 5-9) of corrosion inhibitor CRW2772 dissolved in Acidified methanol: water (90: 10, v/v) has been obtained. MS/MS of spectra of significant ions were acquired in order to provide data that could be used to identify the unknown extracted compounds. The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 were the only significant ions in extracts dissolved in acidified methanol. These fragment to give products ions m/z 212, m/z 240 and m/z 267 respectively as shown in Figure 5-10. These represent the molecular ions of C_{12}, C_{14} and C_{16} which are the series of benzalkonium compounds (quaternary ammonium salts).

Corrosion inhibitor (EC1180) is a commercial oilfield corrosion inhibitor used at the Nasser Oilfield. According to MSDS is stated to contain 1-benzyl-1-(2-hydroxyethyl)-2 coconut oil-2- imidazolinium chloride, benzyl C10-16 alkyldimethyl ammonium chloride, ethylene glycol and isobtanol. Positive ion ES-MS full scan mass spectrum (m/z 50–1850) of corrosion inhibitor EC1180 dissolved in Acidified methanol: water (90: 10, v/v) has been obtained as shown in Figure 5-11. MS/MS spectra of significant ions were acquired and indicate to contain quaternary ammonium compounds as shown in Figure 5-11. This has been shown previously by ESI -MS (Langley et al., 1999). The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 were the only significant ions in extracts dissolved in acidified methanol which was studied produced products ions m/z 212, m/z 240 and m/z 267 respectively as shown in Figure 5-12. These represent the molecular ions of C_{12}, C_{14} and C_{16} which are the series of benzalkonium compounds (quaternary ammonium salts).

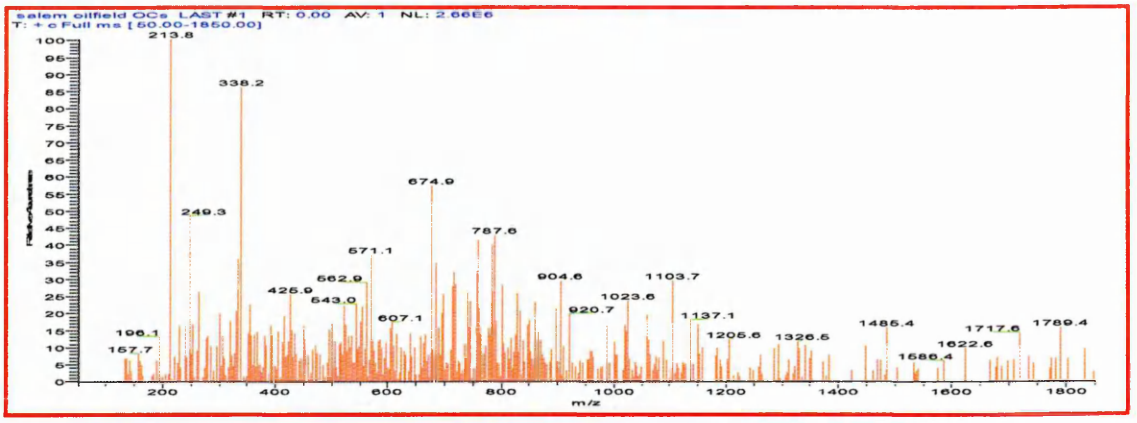


Figure 5-9 ESI mass spectra full scan (m/z 50-1850) of corrosion inhibitor CRW2772

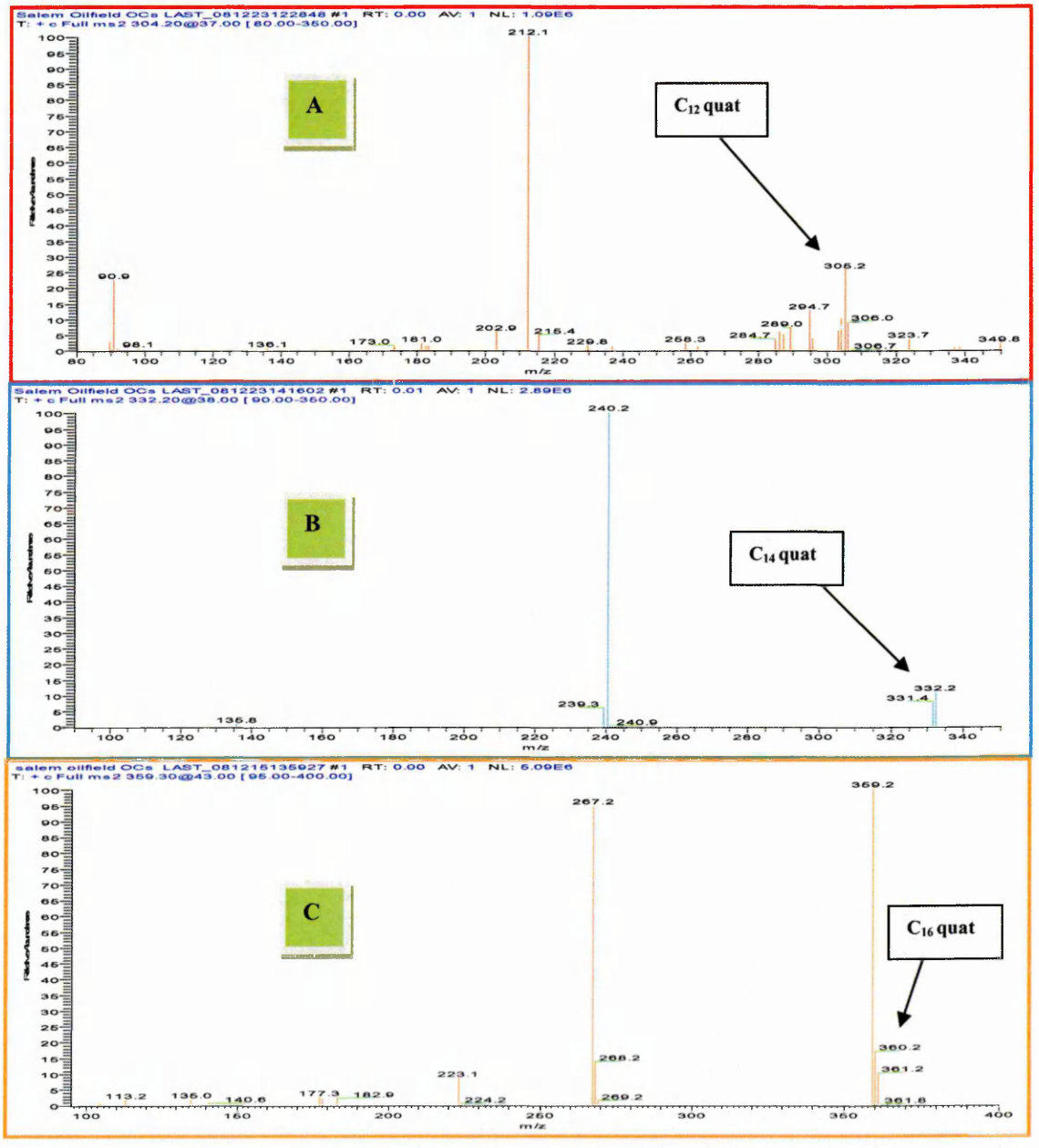


Figure 5-10 ESI mass spectra negative ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 360) benzalkonium quaternary ammonium salts extracted from corrosion inhibitor CRW2772

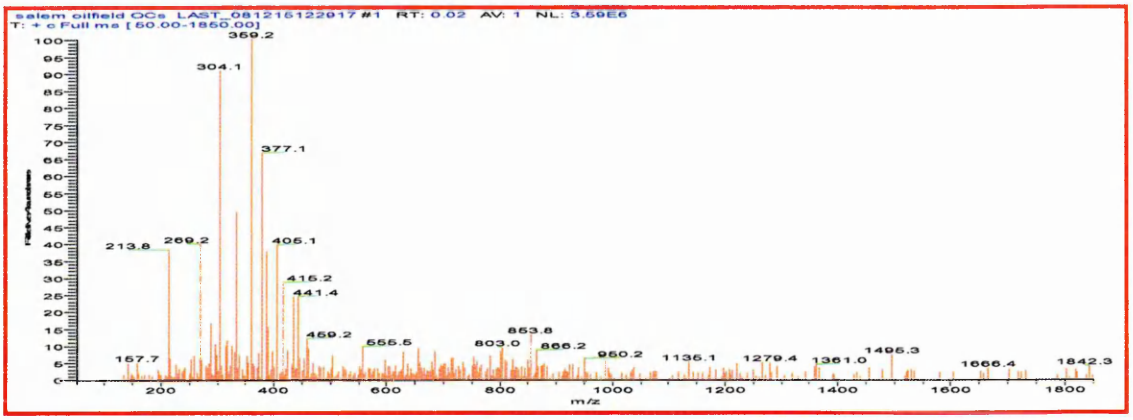


Figure 5-11 ESI mass spectra full scan (m/z 50-1850) of corrosion inhibitor EC1180

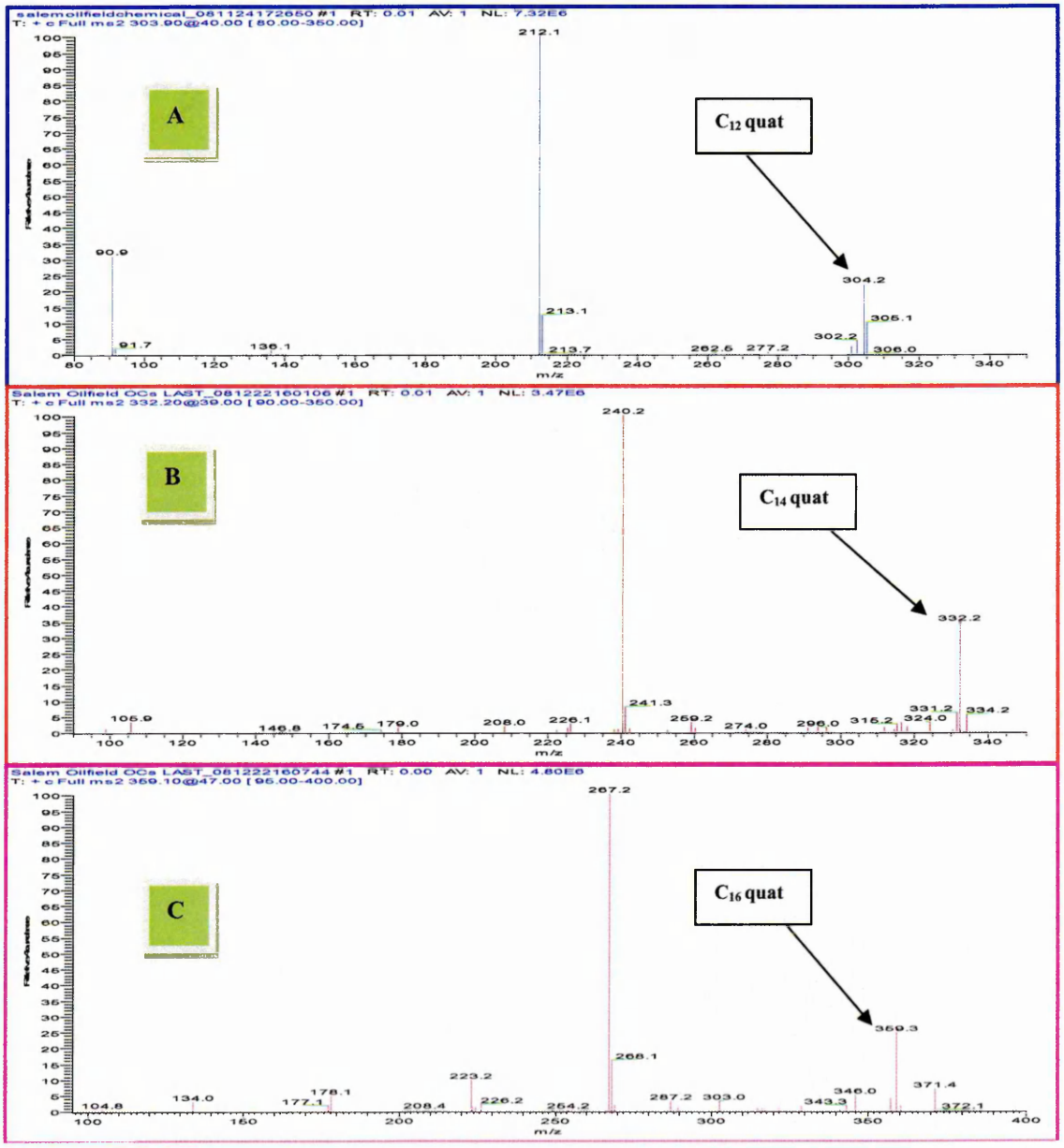


Figure 5-12 ESI mass spectra negative ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 360) benzalkonium quaternary ammonium salts extracted from corrosion inhibitor EC1180

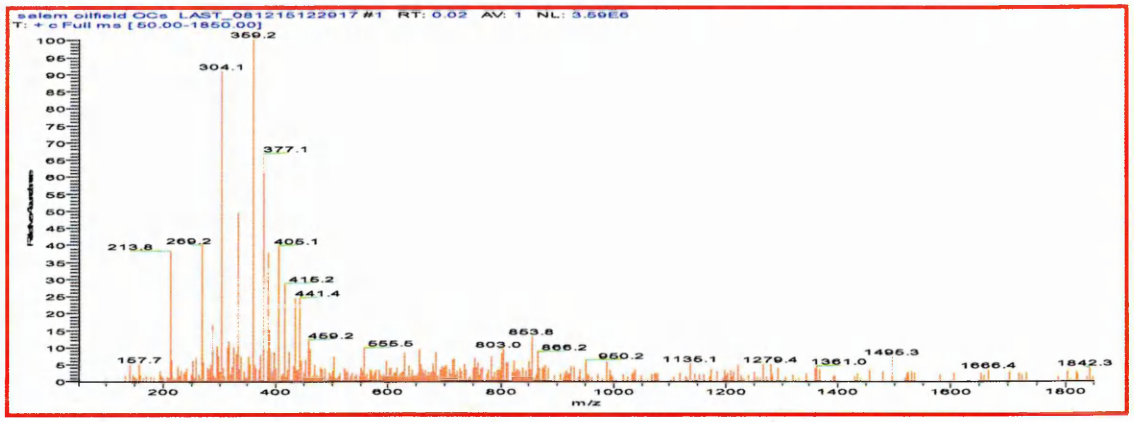


Figure 5-13 ESI mass spectra full scan (m/z 50-1850) of corrosion inhibitor CHIMIC C1053

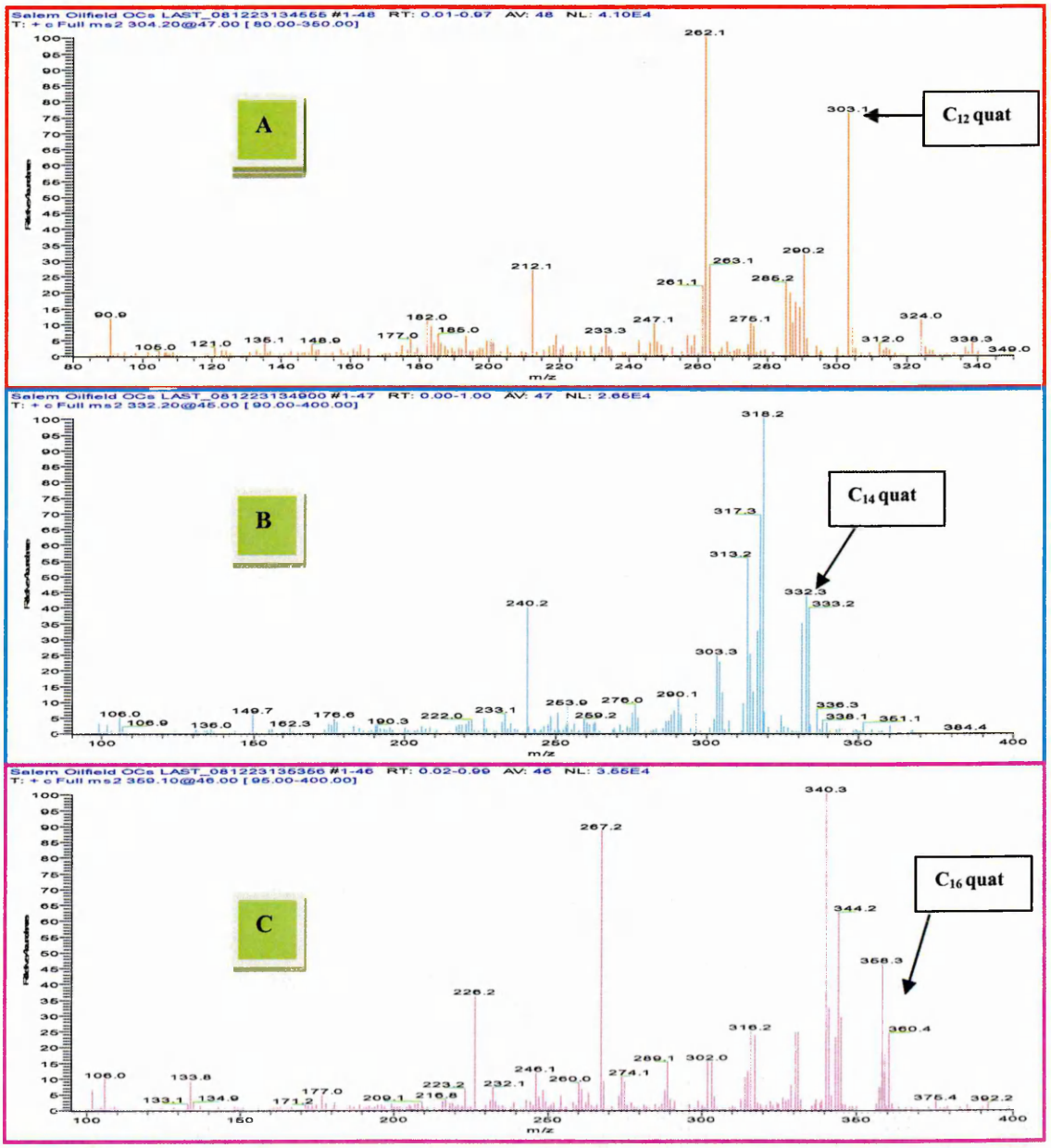


Figure 5-14: ESI mass spectra negative ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 360) benzalkonium quaternary ammonium salts extracted from corrosion inhibitor CHIMIC C1053

Corrosion inhibitor CHIMIC C1053:- is a balanced mixture of compounds used at the Nasser oilfield, and according to MSDS is started to contain a blend of aliphatic amines and amine derivates in high boiling point solvent. A positive ion ES-MS full scan mass spectrum (m/z 50–1850) of corrosion inhibitor CHIMIC C1053 dissolved in acidified methanol: water (90: 10, v/v) has been obtained as shown in Figure 5-13. MS/MS spectra of significant ions were acquired. The spectrum contains at least three series of ions indicative of quaternary ammonium compounds Figure. 5-13, this has been shown previously by ESI -MS (Langley et al., 1999). The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 were the only significant ions in extracts dissolved in acidified methanol which was studied produced product ions m/z 212, m/z 240 and m/z 267 respectively as shown in Figure 5-14. These represent the molecular ions of C_{12}, C_{14} and C_{16} which are the series of benzalkonium compounds (quaternary ammonium salts).

Demulsifier D6261:- is a commercial emulsion breaker and it is blend of alkoxylate resins and high molecular weight polyols in an aromatic solvent. According to the MSDS is state to contain, aromatic solvent, kerosene, block polymer, amine oxalkyate and cationic surfactant. A positive ion ES-MS full scan mass spectrum (m/z 50–1850) of Demulsifier D6261 has been obtained. MS/MS spectra of significant ions were acquired. The spectrum is clearly having some similar m/z value to those seen in corrosion inhibitors. A series of ions indicates to contain QACs as shown in Figure. 5-15. The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 were the only significant ions in extracts from demulsifier and dissolved in acidified methanol which was studied produced fragment to give a common products ions m/z 212, m/z 240 and m/z 267 respectively and these represent the molecular ions of C_{12}, C_{14} and C_{16} which are the series of benzalkonium compounds (quaternary ammonium salts) as shown in Figure 5-16.

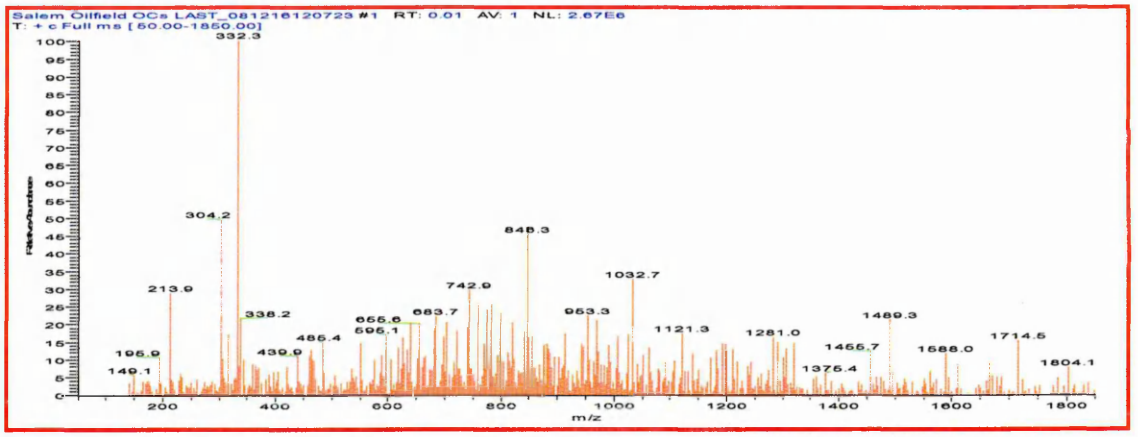


Fig. 5-15 ESI mass spectra full scan (50-1850 m/z) of demulsifier inhibitor D6261

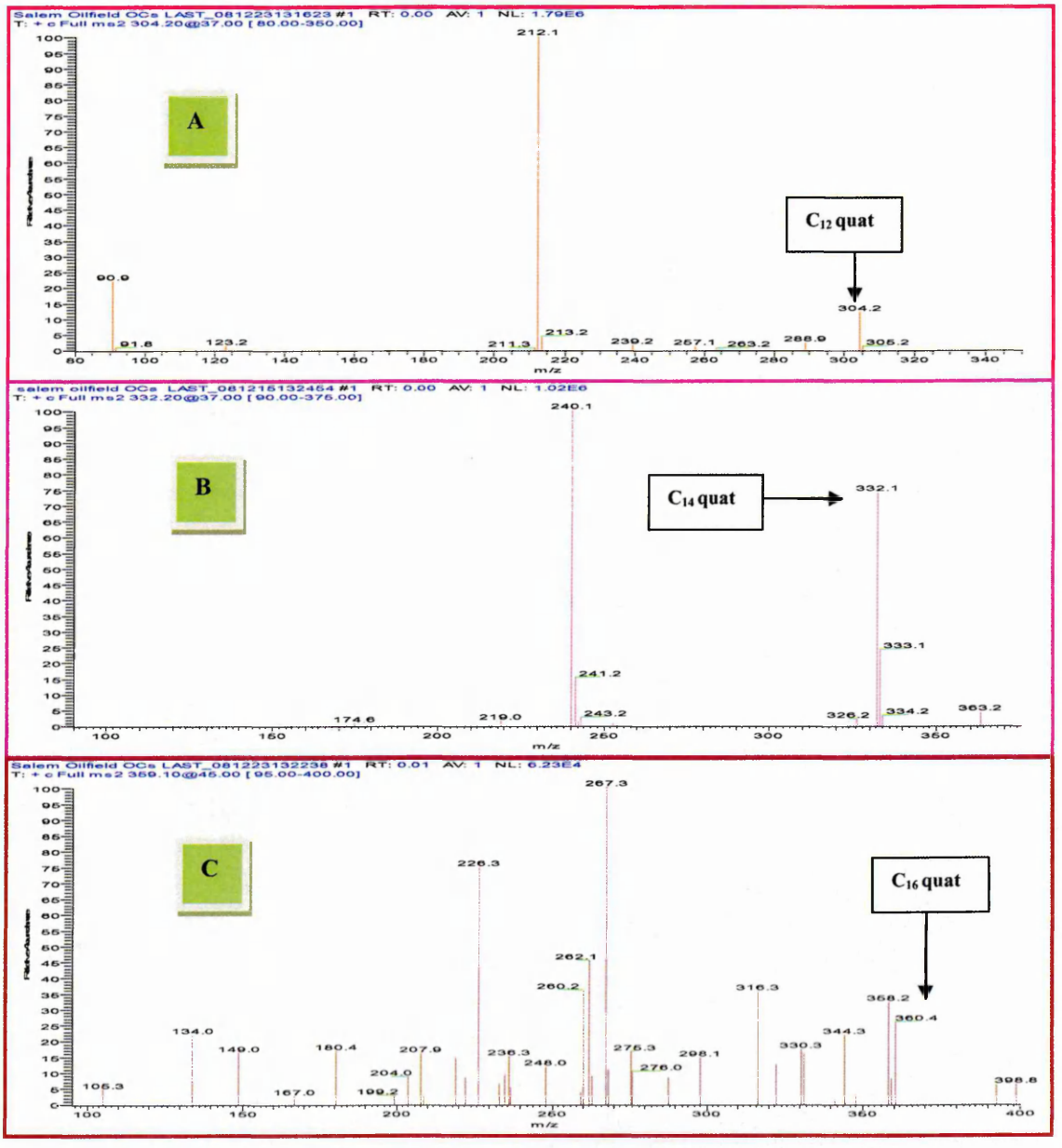


Figure 5-16 ESI mass spectra negative ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 360) benzalkonium quaternary ammonium salts extracted from demulsifier D6261

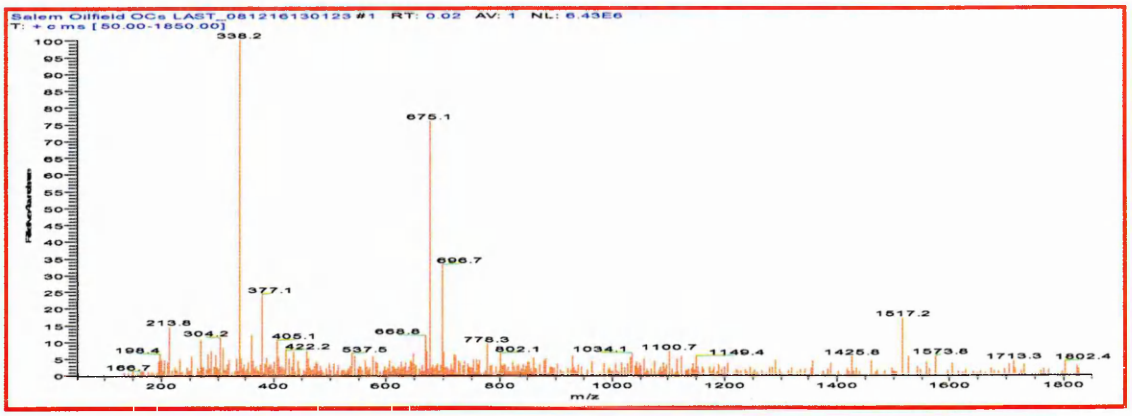


Figure 5-17 ESI mass spectra full scan (m/z 50-1850) of mixed scale inhibitors (SCW2510 and EC6165)

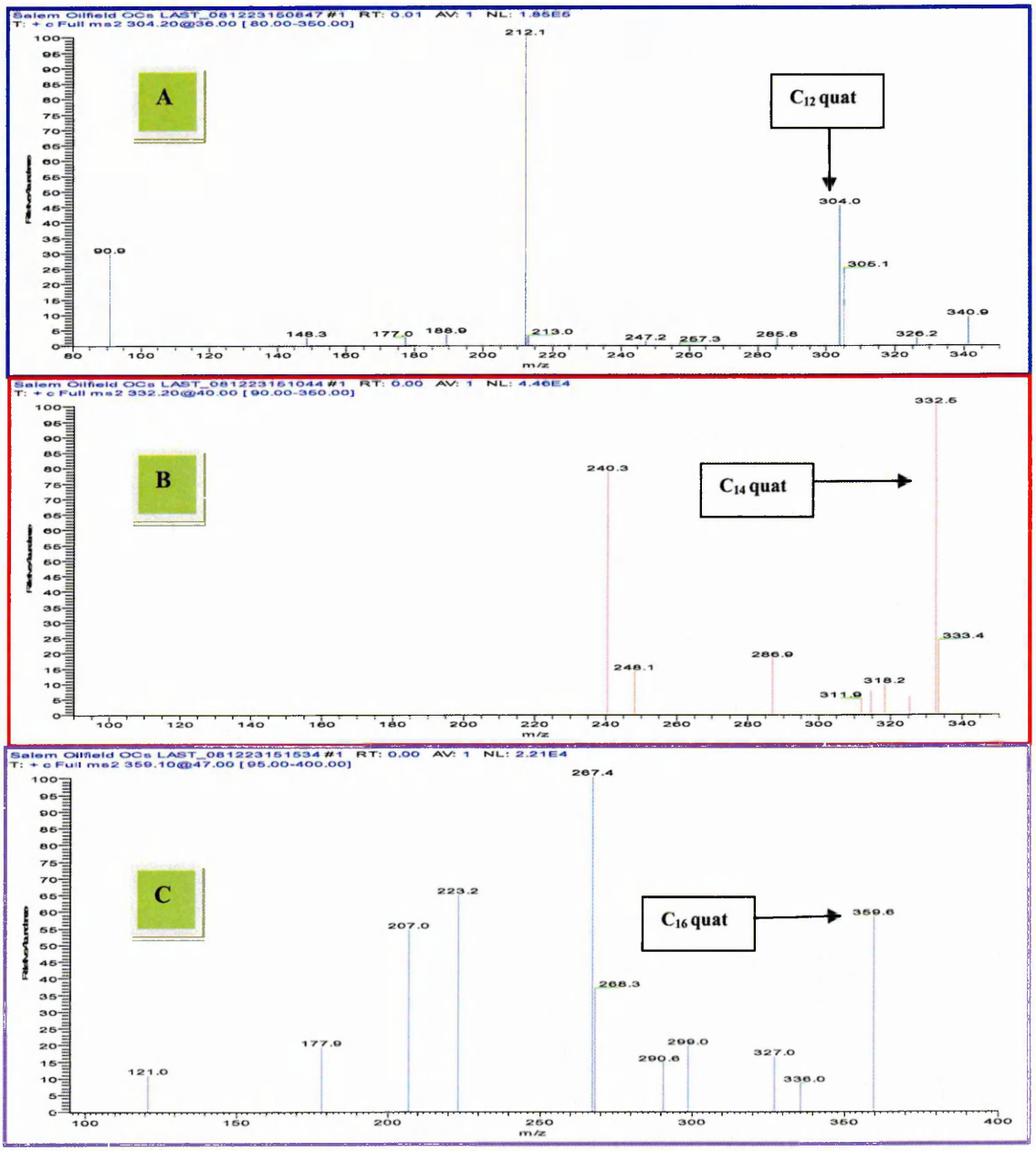


Figure 5-18 ESI mass spectra negative ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C16 (m/z 360) benzalkonium quaternary ammonium salts extracted from mixed scale inhibitors (SCW2510 and EC6165)

Scale inhibitors SCW2510 and EC6165:- is a commercial oilfield scale inhibitors usually used in the Nasser oilfield. It is started to contain (MSDS) phosphonate, a neutralised polyacrylate, ethylene glycol, alkanolamine phosphate and phosphoric acid. A positive ion ES full scan mass spectrum (m/z 50–1850) of a mixture of SCW2510 and EC6165 dissolved in acidified methanol: water (90:10 v/v) has been obtained of significant ions were acquired, Figure 5-17. The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 were the only significant ions in extracts from scale inhibitors (SCW2510 and EC6165) which were studied produced fragment to give a common products ions m/z 212, m/z 240 and m/z 267 respectively, confirming that these represent the molecular ions of C_{12}, C_{14} and C_{16} benzalkonium compounds as shown in Figure 5-18.

Corrosion - Biocide inhibitors EC6206A:- is a commercial biocide inhibitor used at the studied oilfield and it is started to contain (MSDS) 1-benzyl-1-(2-hydroxyethyl)-2 coconut oil-2- imidazolinium chloride, benzyl C10-16 alkyldimethyl ammonium chloride, ethylene glycol and isobtanol. A positive ion ES-MS full scan mass spectrum (m/z 50–1850) of EC6206A has been obtained of significant ions was acquired and is clearly having some similar m/z value to those seen in corrosion inhibitors and indicates to contain quaternary ammonium compounds (Figure 5-19). The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 produced fragment to give a common products ions m/z 212, m/z 240 and m/z 267 respectively as shown in Figure 5-20 which are identical to the standards. These represent the molecular ions of C_{12}, C_{14} and C_{16} which are the series of benzalkonium compounds.

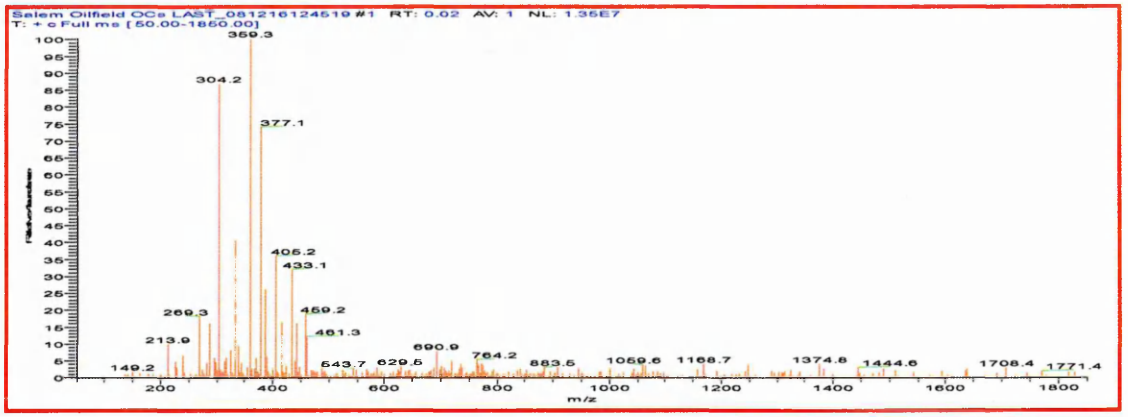


Figure 5-19 ESI mass spectra full scan (m/z 50-1850) of corrosion - biocide inhibitors EC6206A

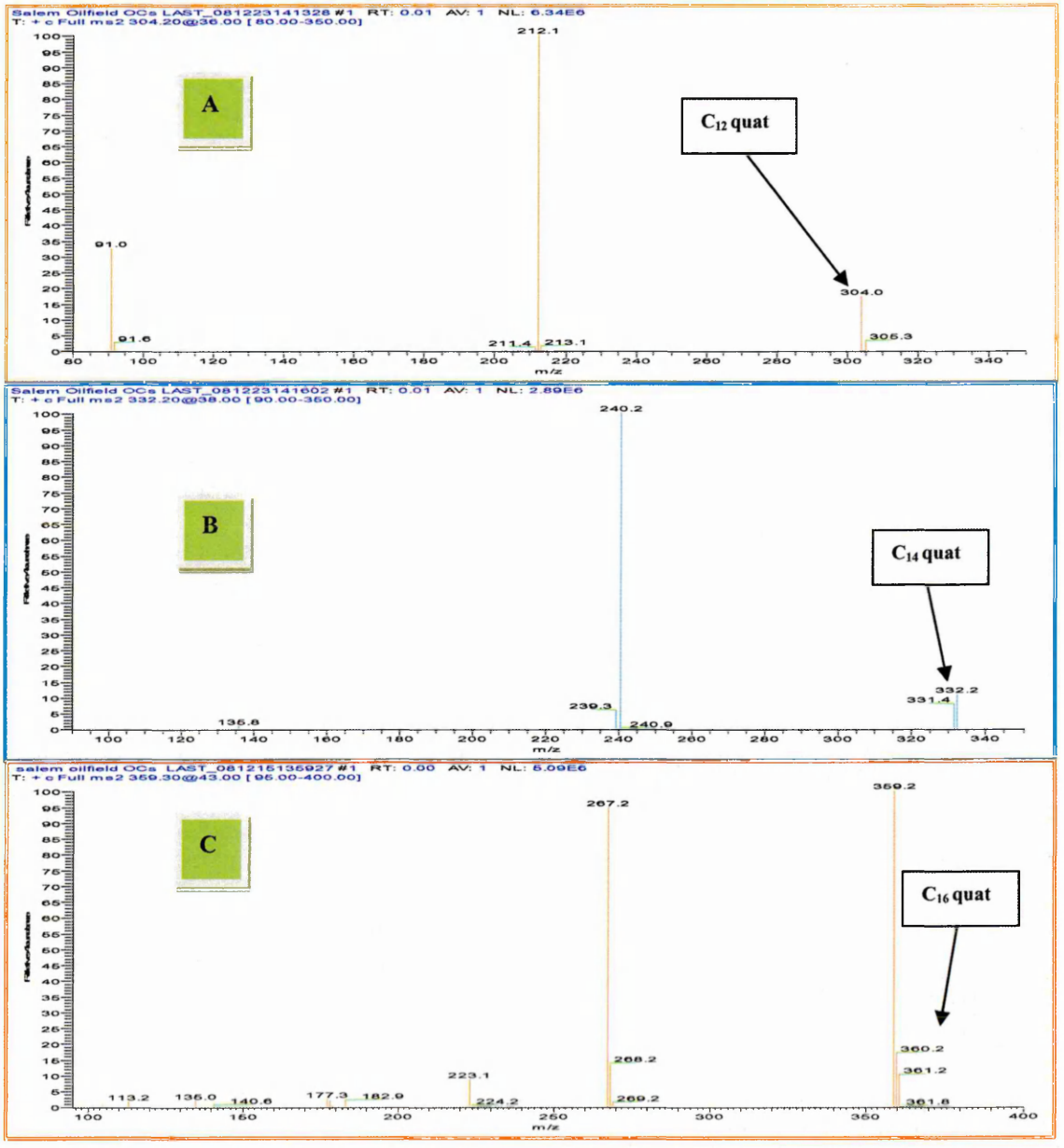


Figure 5-20 ESI mass spectra negative ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 360) benzalkonium quaternary ammonium salts extracted from corrosion - biocide inhibitors EC6206A

5.4.1.2 Qualitative analysis of oilfield chemicals in soil and produced water samples by ESI-MS

OFCs are a structurally diverse range of chemicals that are applied to the production fluids at oilfields to help oil water separation and to reduce the effects of corrosion and scale deposition. Most of their chemistry is amine based, although a few involve phosphorus chemistry (Langley et al., 1999). These oilfield chemicals will be discharged to the open environment associated with PW. PW and soil were investigated for OFCs, DCM extracts were used and then the compounds of interest were then concentrated from the aqueous solution by use of reversed phase C18 SPE columns. Then the extracts were re-dissolved in acidified MeOH and analyzed by ESI-MS and LC-ESI-MS. Positive ion full scan spectra (m/z 50-1850), zoom scans (ZS) of significant ions were acquired and the results were as follows:

The methanol used as solvent was directly injected into the ESI-MS in order to investigate the blank response and to confirm that the solvent did not contain any ions of interest. Figure 5-21 shows the full scan spectra of pure methanol used as solvent obtained by ESI– MS was found not to contain any of the ions m/z 304.4, 332.2 and 360.2 which are the significant ions found in the spectra of a standard. A positive ion ESI-MS full scan mass spectrum (m/z 50–1850) of the acidified MeOH spiked with mixed OFCs used at the Nasser oilfield as standard were also subjected to ESI-MS as shown in Figure 5-22, it is clearly having similar m/z value to those seen in pure standards and commercial OFCs and this is to confirm that the major ions m/z 304.4, 332.2 and 360.2 similar to those ions found in the standards used at the site and not from the solvents which were used for sample preparation. The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 produced fragment to give a common products ions m/z 212, m/z 240 and m/z 267 respectively as shown in Figure 5-23 which are identical to the pure standards alkylbenzyldimethylammonium chlorides. These represent the molecular ions of C_{12}, C_{14} and C_{16} which are the series of benzalkonium compounds which is the same as found in the standards.

The OFCs extracted from PW were subjected to ESI-MS under similar conditions to the standards. A positive ion ESI-MS full scan mass spectrum (m/z 50–1850) of the isolated compounds in acidified MeOH extracts from PW has been obtained of significant ions were acquired. The full scan analysis of oilfield chemicals (OFCs) in PW is shown in Figure 5-24, the precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 were the only significant ions in extracts from produced water which are investigated. MS^2 analysis of precursor ions m/z 304.4, 332.2 and 360.2 produced

fragment to give a common products ions m/z 212, m/z 240 and m/z 267 respectively, this spectrum showed that the same ions (304.4, 332.2 and 360.2) found in the standards and these represent the molecular ions of C_{12}, C_{14} and C_{16} benzalkonium compounds. PW samples were found to contain residual C_{12}, C_{14} and C_{16} quaternary ammonium salts (quats) as shown in as shown in Figure 5-25.

The soil samples collected from the Nasser oilfield were analyzed for OFCs by using ESI-MS under similar conditions as the standards. Figure 5-26 shows the positive ion full scan spectrum (m/z 50–1850) of the compounds extracted from polluted soil collected from the study site. The precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 produced fragment to give the products ions m/z 212, m/z 240 and m/z 267 respectively as shown in Figure 5-27, these spectrum showed that the same ions (304.4, 332.2 and 360.2) found in the standards and these represent the molecular ions of C_{12}, C_{14} and C_{16} benzalkonium compounds. The analysis of OFCs in soil samples it is more complicated compared to the PW samples and this is due to high salt and hydrocarbon concentrations. However, LC-ESI-MS analysis overcomes these problems by monitoring the molecular ions of the target molecules during chromatographic analysis (Langley et al., 1999). A comparison between the precursor ion peaks on ESI-MS^2 analysis of ions at m/z 304.4, 332.2 and 360.2 for the OFCs standards Figures (5-8, 5-10, 5-12, 5-14, 5-16, 5-18 and 5-20), PW samples Figure 5-25 and polluted soil samples Figure 5-27 indicates similar major ions m/z 304.4, 332.2 and 360.2 to those detected in oilfield chemicals standards (which represent the molecular ions of C_{12} and C_{14}, C_{16} alkyl chain components). This gives clear evidence that the benzalkonium quaternary ammonium salts (corrosion inhibitor) were detected in soil and PW; these are considered one of contaminants in produced water according to Grigson et al., 2000 these oilfield chemicals are completely degraded under laboratory conditions.

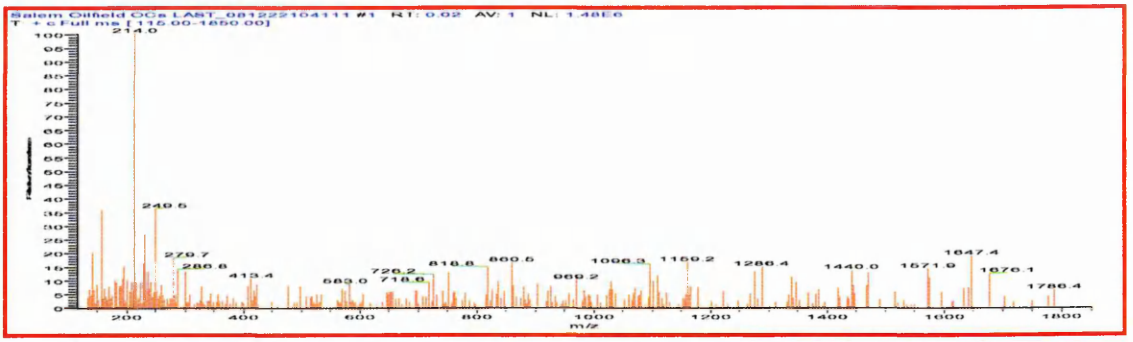


Figure 5-21 ESI mass spectra full scan (m/z 50-1850) of acidified methanol

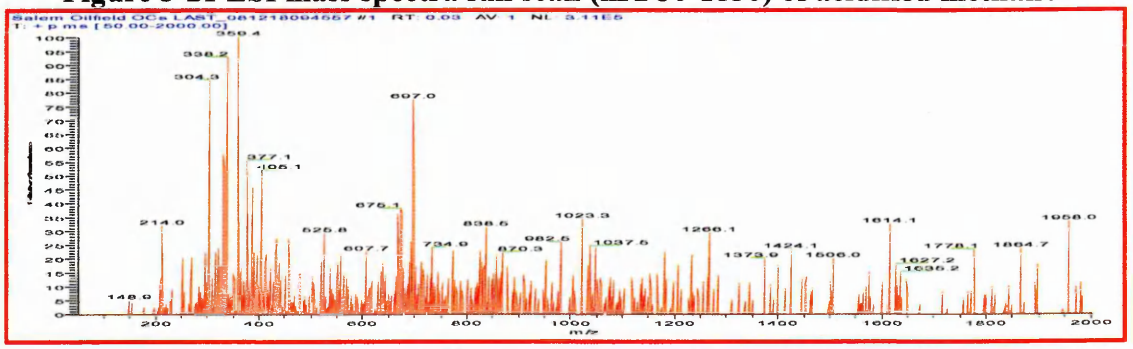


Figure 5-22 ESI mass spectra full scan (m/z 50-1850) of acidified methanol spiked with corrosion inhibitor

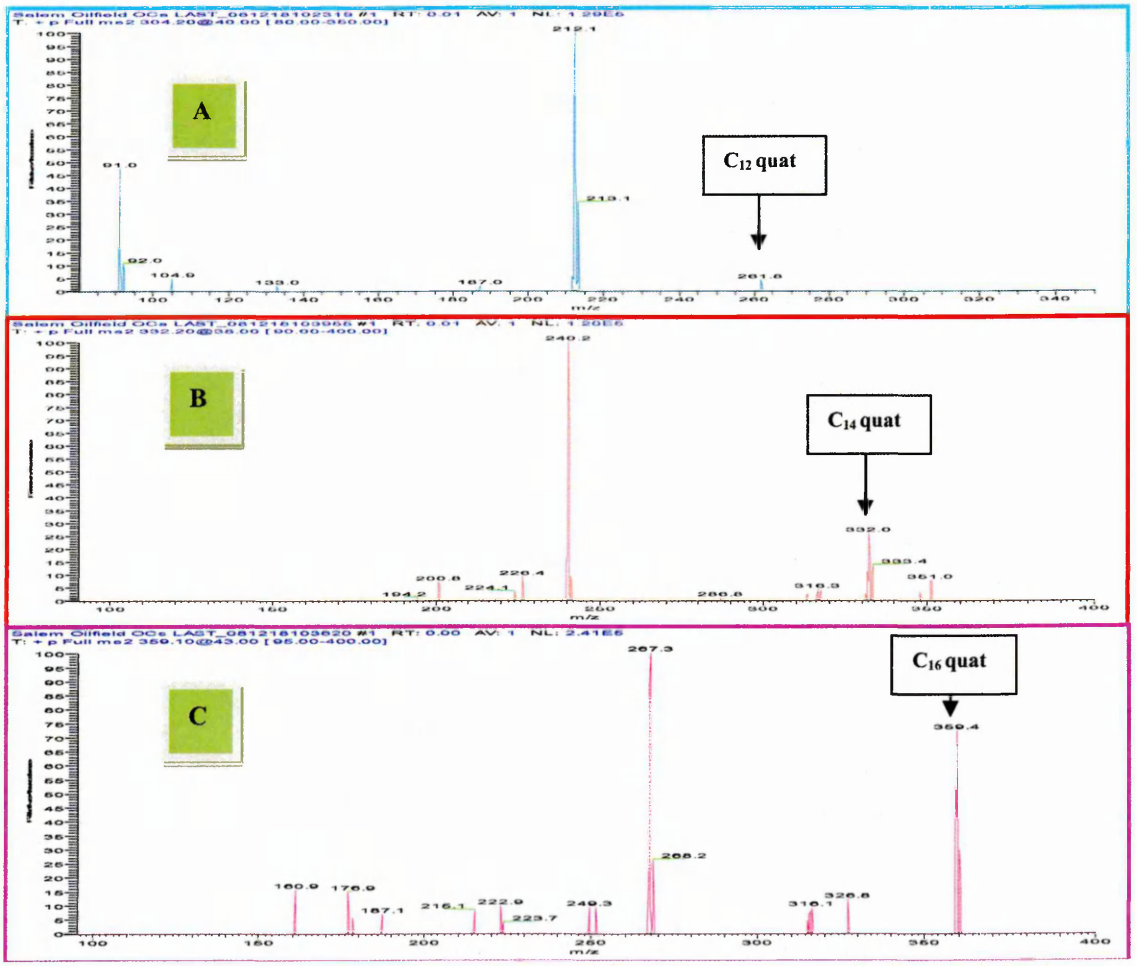


Figure 5-23 ESI mass-mass spectra ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 360) benzalkonium quaternary ammonium salts extracted from spiked methanol with standard chemicals

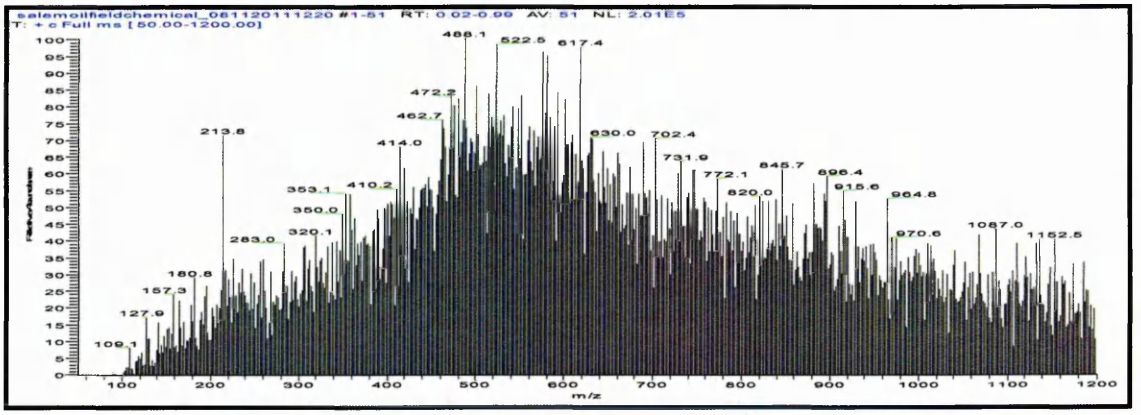


Figure 5-24 ESI mass spectra full scan (m/z 50-1850) of produced water

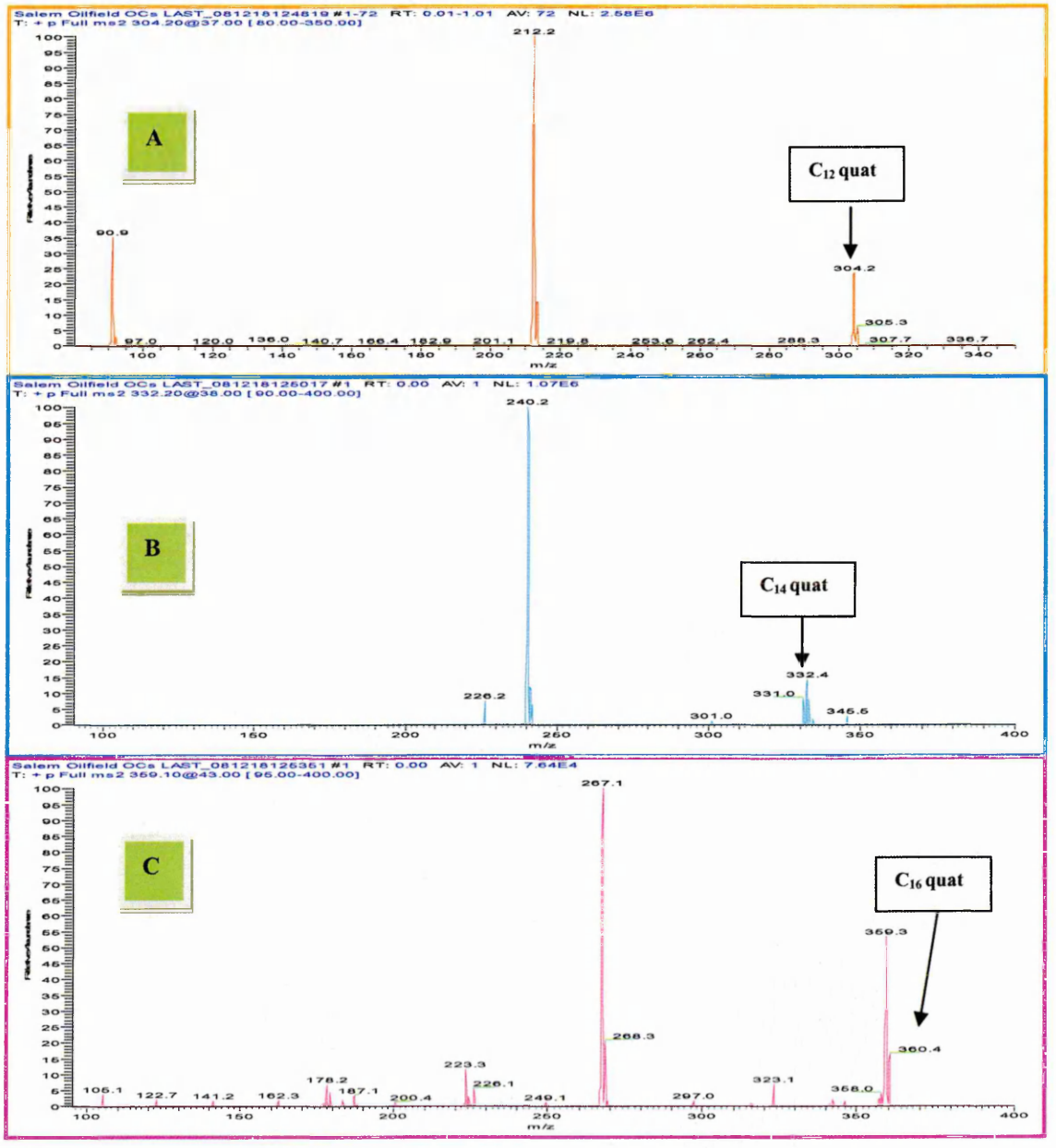


Figure 5-25 ESI mass-mass spectra of ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 359) benzalkonium quaternary ammonium salts extracted from produced water

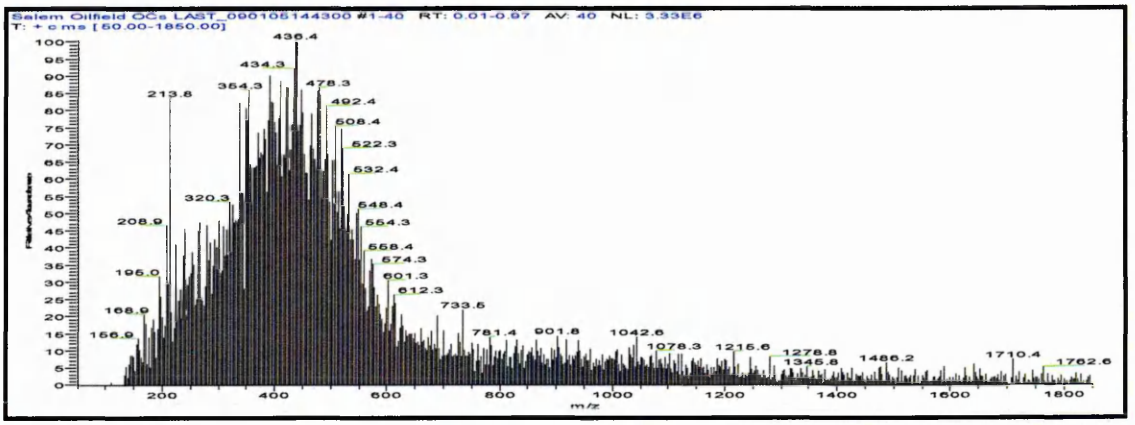


Figure 5-26 ESI mass spectra full scan (m/z 50-1850) of contaminated soil sample

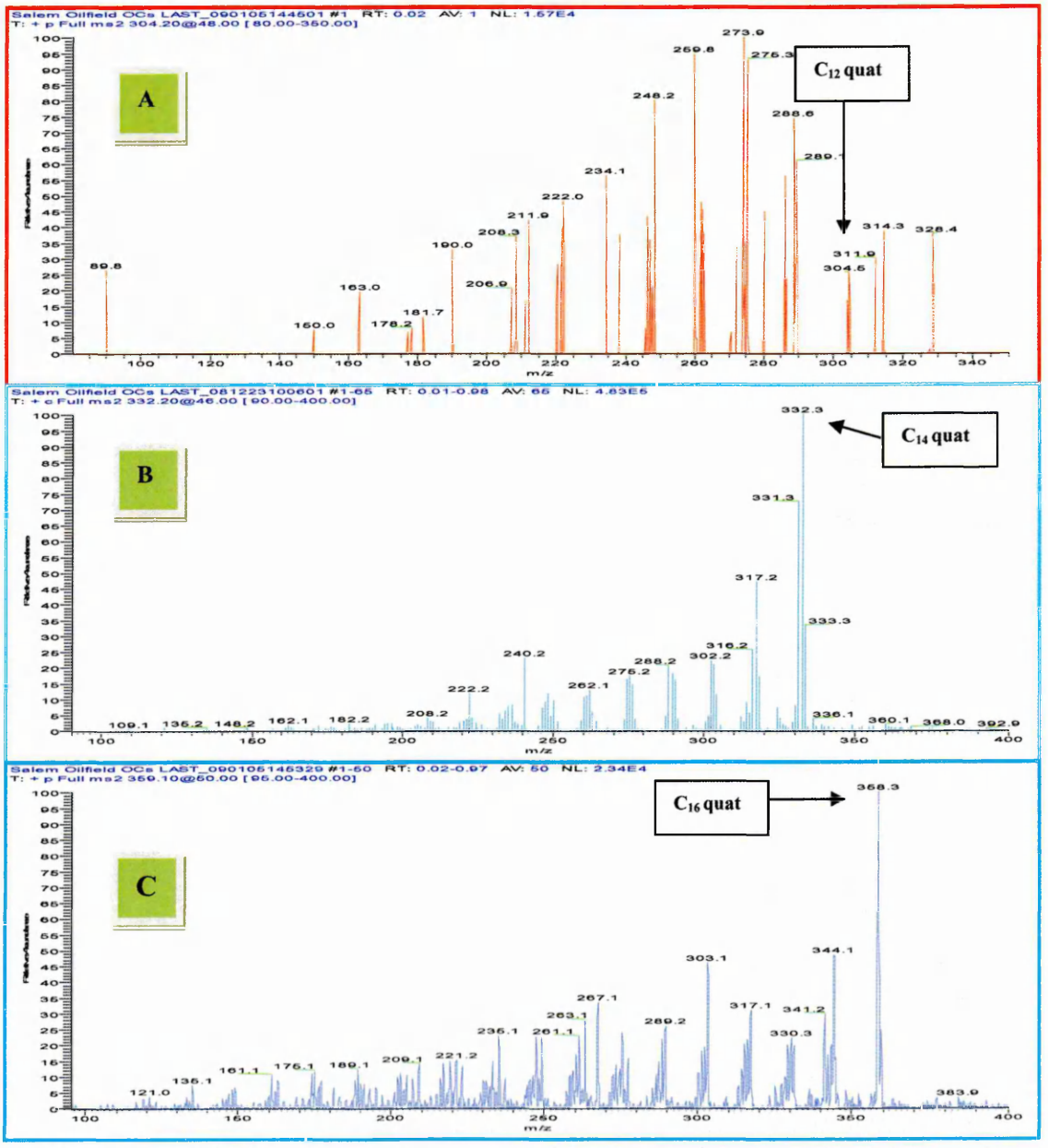


Figure 5-27 ESI mass-mass spectra ion peaks labelled are A) C_{12} (m/z 304), B) C_{14} (m/z 332) and C) C_{16} (m/z 359) benzalkonium quaternary ammonium salts extracted from soil sample

5.4.2 Qualitative analysis of oilfield chemicals by LC-ESI-MS technique

The introduction of ESI-MS has seen the application of the technique to the identification of a growing number of polar chemicals in environmental and industrial matrices. The use of ESI-MS has great potential for the determination of OFCs in environmental samples (Langley et al., 1999). The presence of interfering peaks, from the sample matrix made the use of ESI-MS coupled with LC necessary for the determination of the OFCs in environmental samples (Langley et al., 1999 and McCormack et al., 2002). However, the development of a LC method should make it possible to separate any interfering peaks, due to the matrix from the OFCs. This should be achievable with the LC equipped with the ESI-MS and using a suitable column and mobile phase. MS coupling to the chromatographic techniques has always been desirable due to the sensitive and highly specific nature of MS compared with other chromatographic detectors (Langley et al., 1999 and McCormack et al., 2002).

A method was developed which allowed the separation of individual compounds by reversed phase LC with detection by ESI. At first mixed commercial OFCs standard solutions were subjected to LC-ESI-MS the mass chromatograms obtained for m/z range 303-305, 331-331, 359-361 and 387-389 corresponded to the observed ion peaks of m/z 304, 332, 360 and 388 which represent the molecular ions of C_{12}, C_{14}, C_{16} and C_{18} as shown in Figure 5-28, these data obtain from the mixed commercial OFCs standards solution will be used to identify the unknown extracted compounds in PW and soil samples. In order to investigate the blank response the solvent used in the sample extraction (acidified methanol) was subjected to LC-ESI-MS under the same conditions as the mixed commercial OFCs standards solution. The chromatograms obtained for acidified methanol found not to contain any of the ions (i.e. m/z 304, 332, 360 and 388) which represent the molecular ions of C_{12}, C_{14}, C_{16} and C_{18} as shown in Figure 5-29.

LC-ES-MS analysis of tap water spiked with mixed commercial OFCs standard solutions is shown in Figure 5-30, and the major ions m/z 304.4, 332.2 and 360.2 similar to those ions found in the mixed commercial OFCs standards solution used at the Nasser oilfield were detected.Residues of OFCs extracted from polluted soil and PW samples analysed by ES-MS were also subjected to LC/ MS coupled to ES/MS under similar conditions as commercial OFCs standard. The results showed that PW and soil samples gave peaks corresponding to suspected quaternary ammonium compounds of alkyl chain lengths identical to the commercial standards Figures 5-31, 5-32, 5-33, 5-34 and 5-35. Clearly, this is reliable evidence that these chemicals were found in soil and PW collected from the study oilfield.

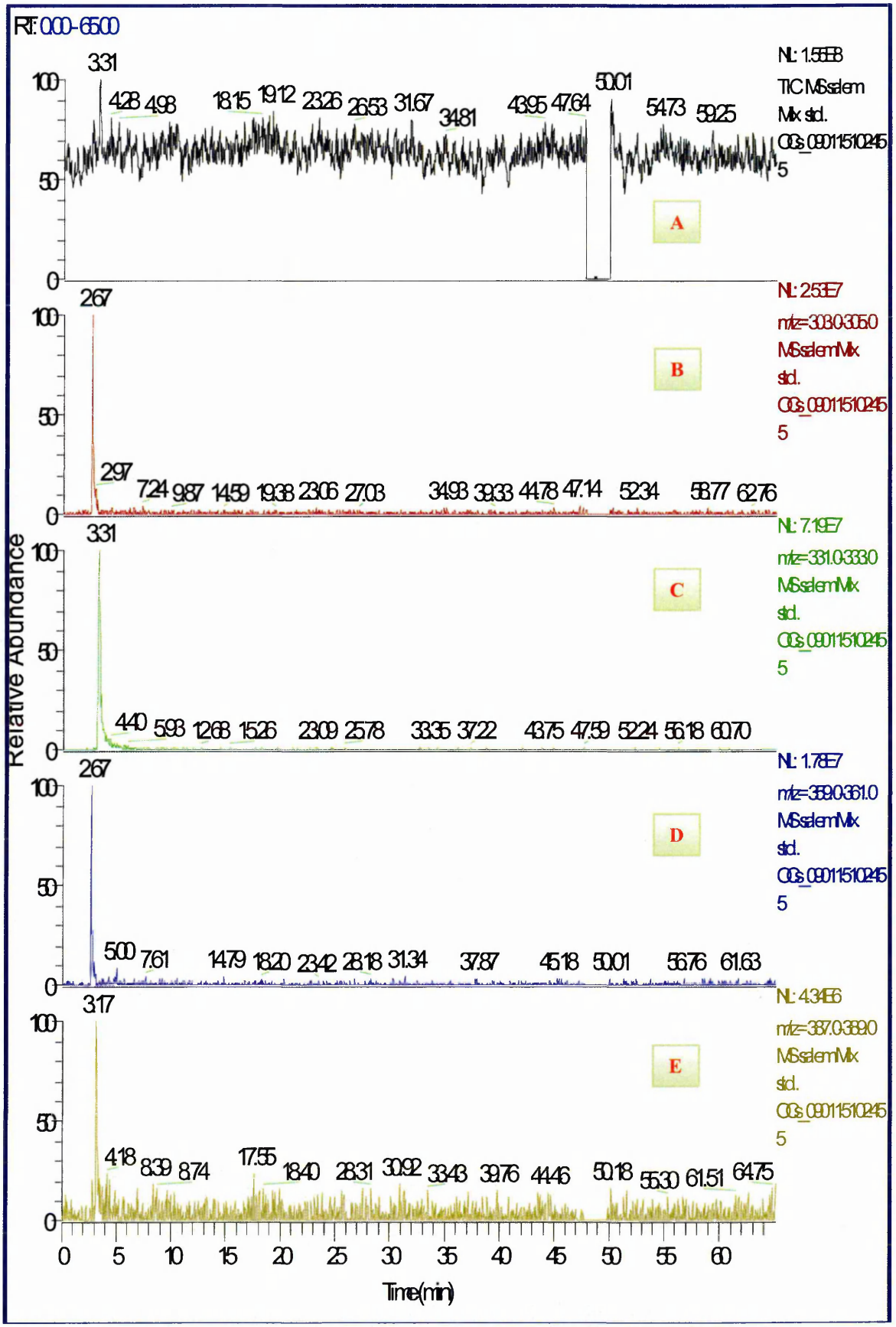


Figure 5-28 ESI extracted ion chromatograms of quaternary ammonium compounds obtained by LC/ESI-MS analysis of mixed standard (A) full Scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

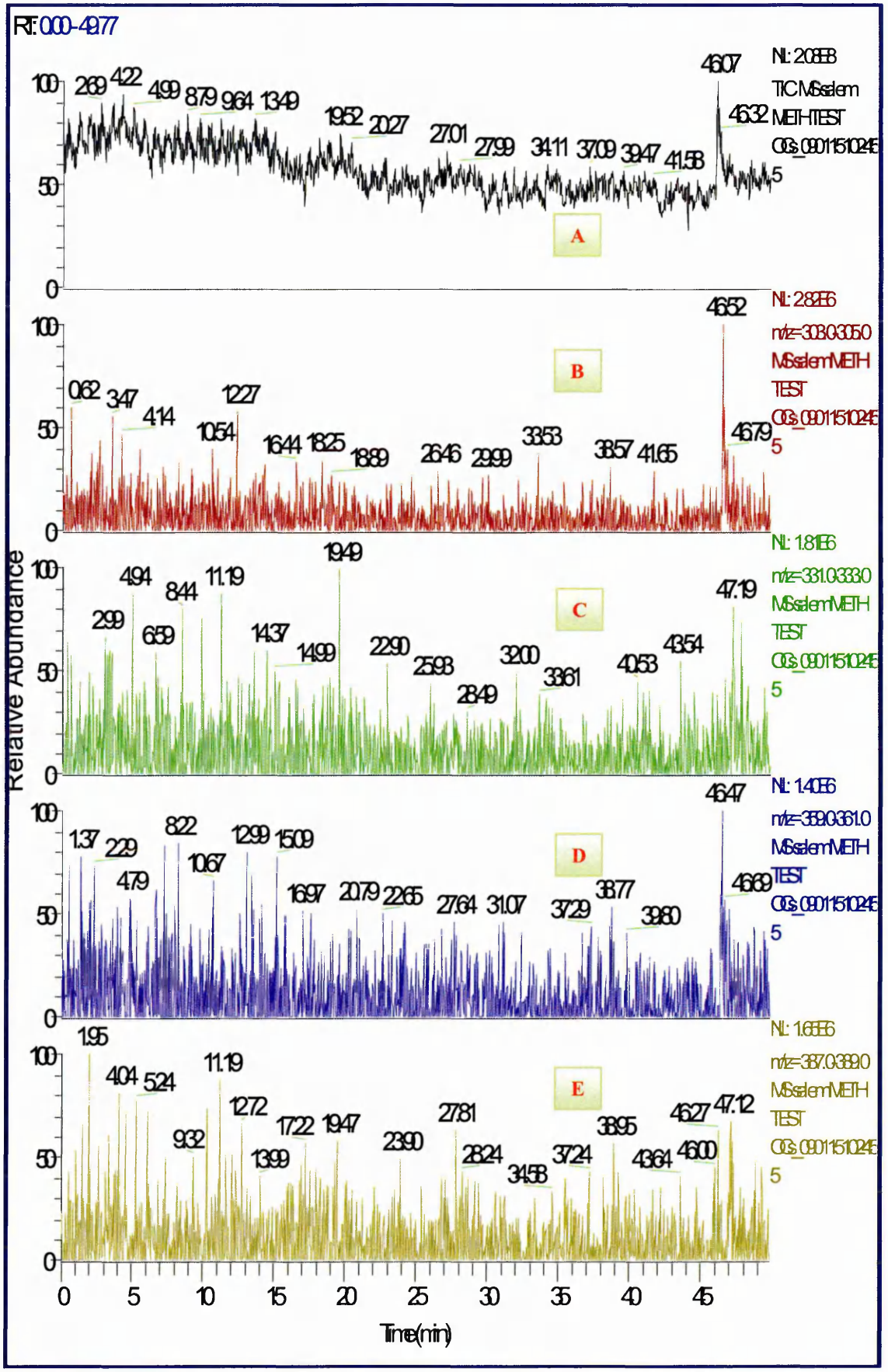


Figure 5-29 ESI extracted ion chromatograms of acidified methanol by LC/ESI-MS analysis (A) full Scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

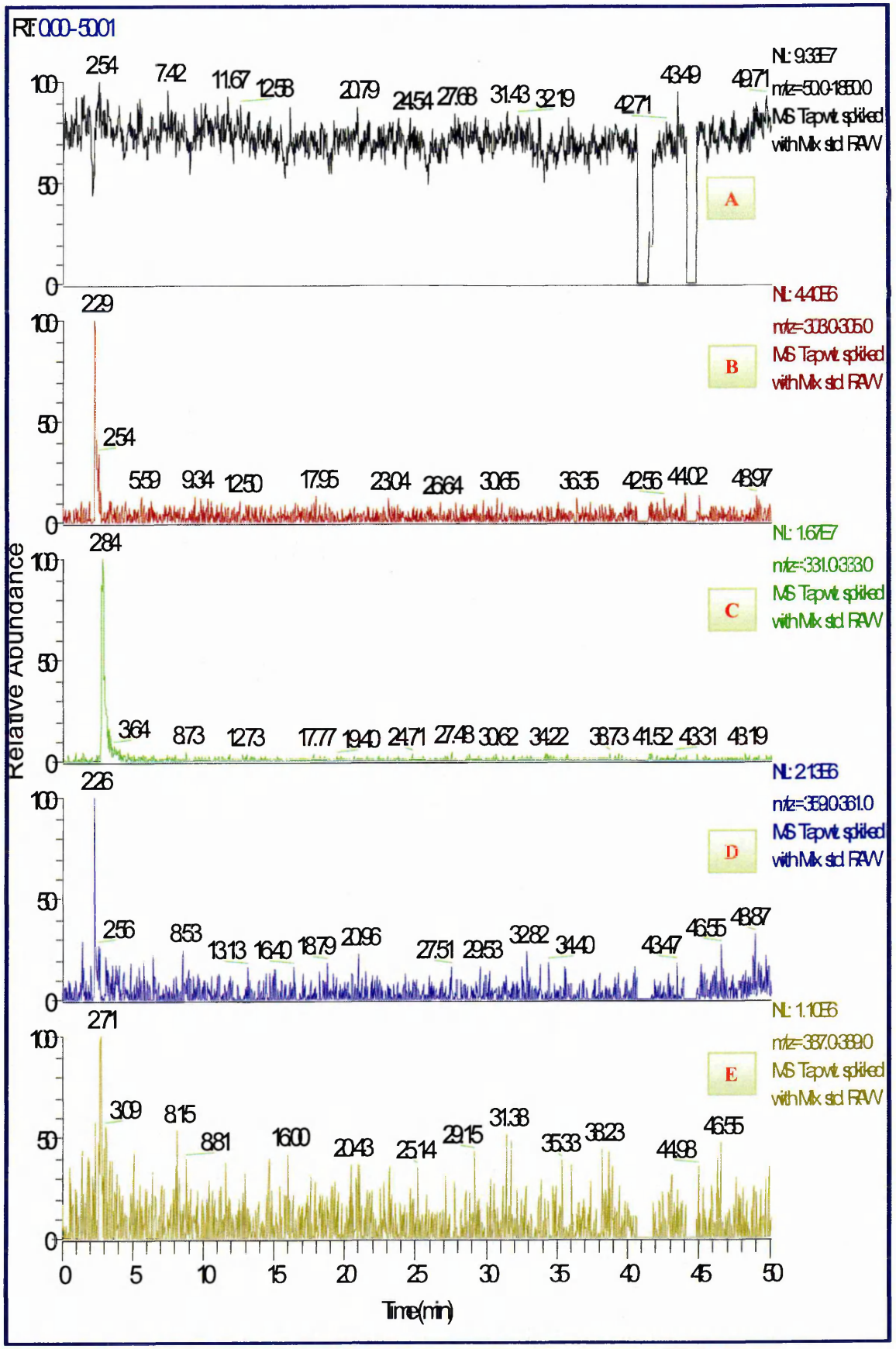


Figure 5-30 ESI extracted ion chromatograms of quaternary ammonium compounds obtained by LC/ESI-MS analysis of tap water spiked with mixed standard (A) full Scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

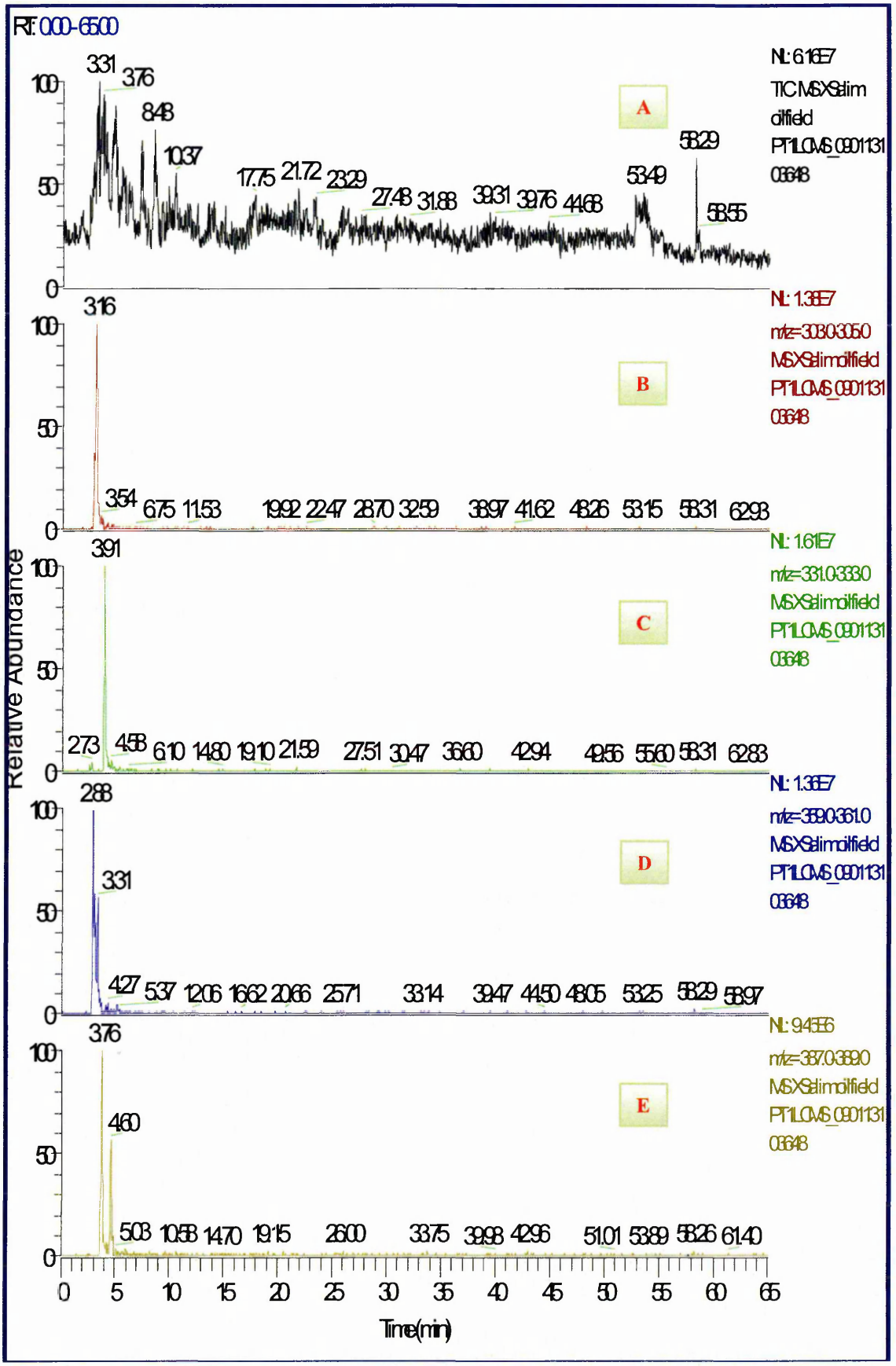


Figure 5-31 ESI extracted ion chromatograms of quaternary ammonium compounds obtained by LC/ESI-MS analysis of produced water sample (SPT1). (A) Full scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

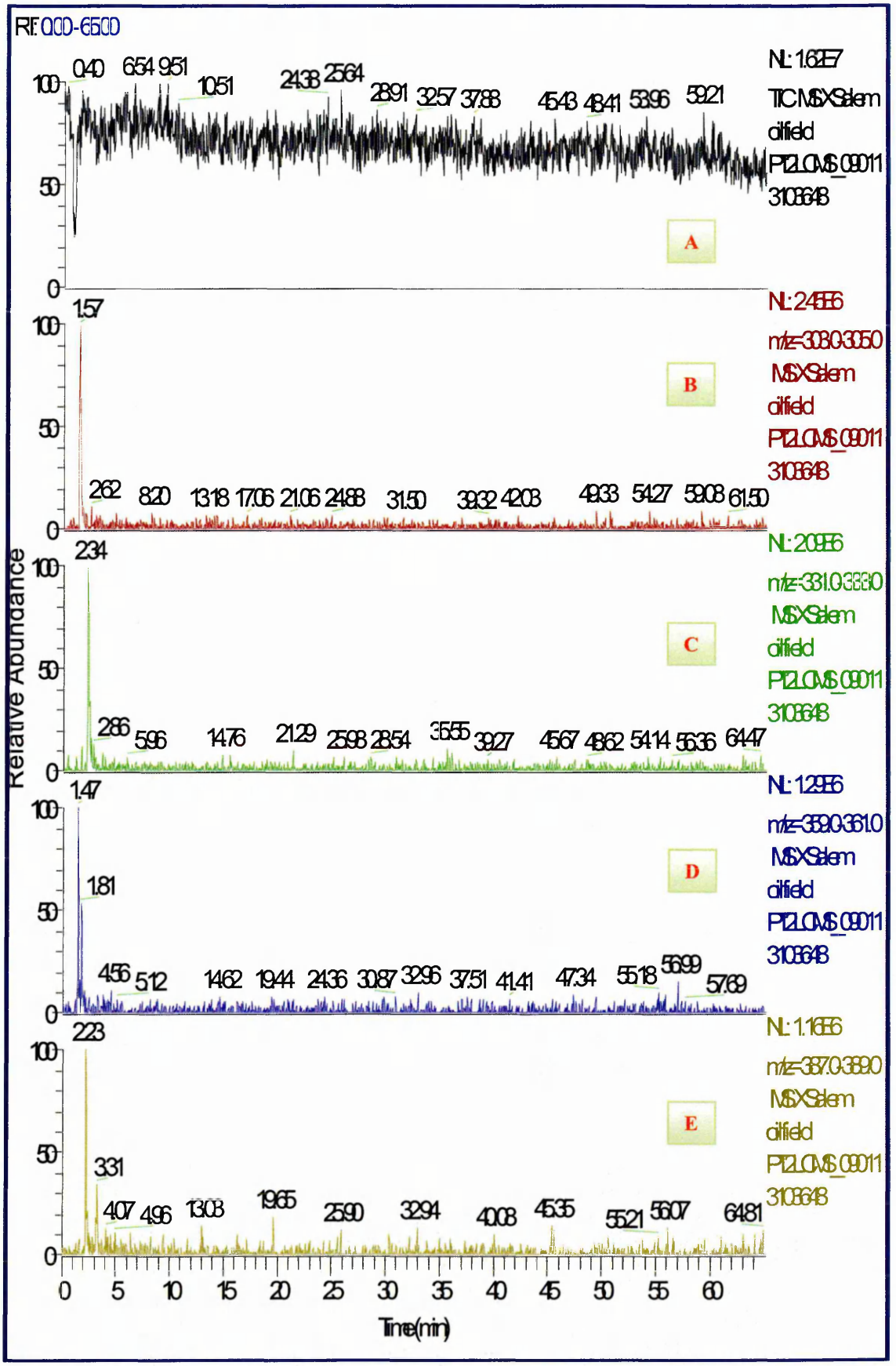


Figure 5-32 ESI extracted ion chromatograms of quaternary ammonium compounds obtained by LC/ESI-MS analysis of produced water sample (SPT2). (A) Full scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

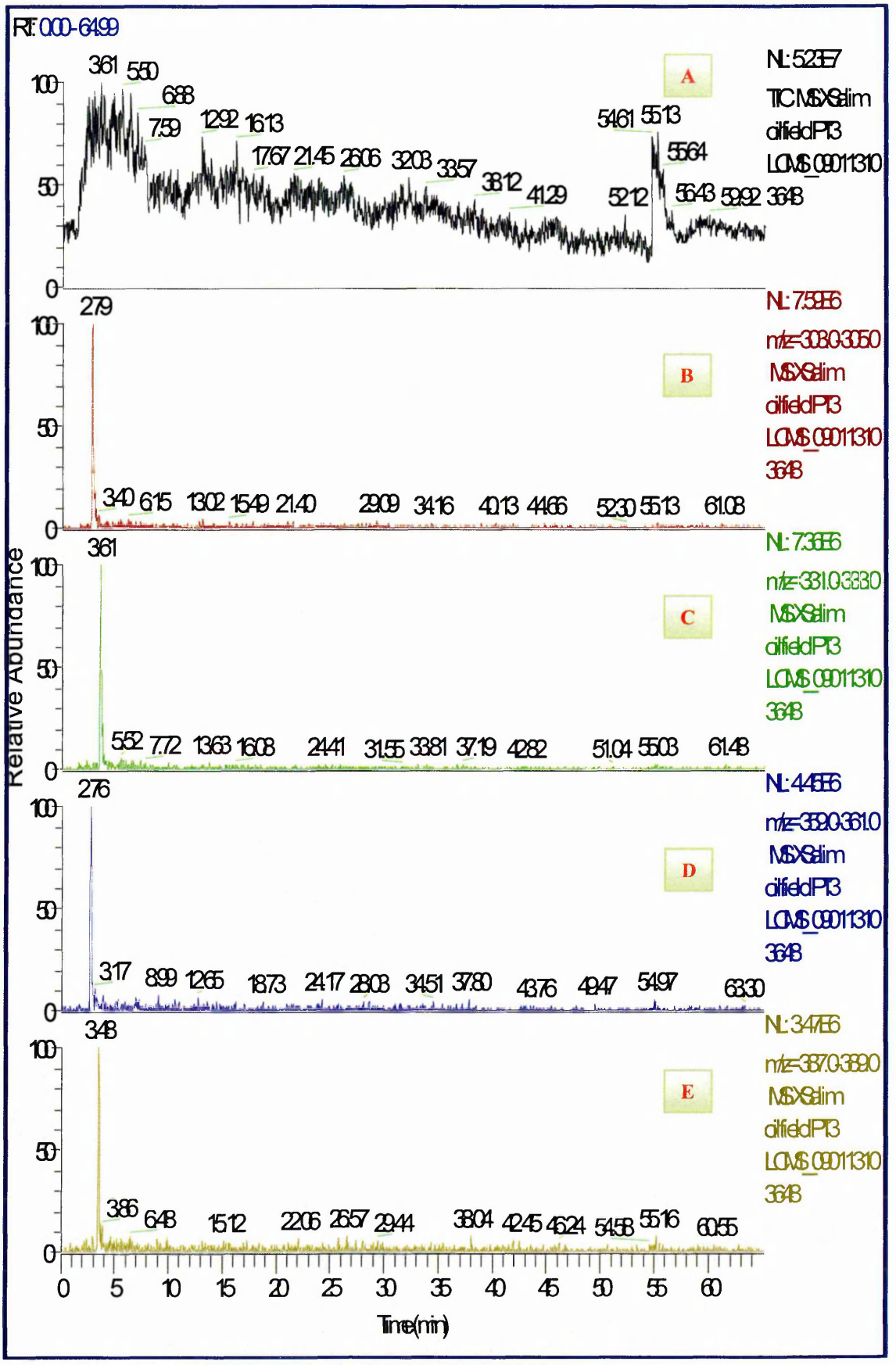


Figure 5-33 ESI extracted ion chromatograms of quaternary ammonium compounds obtained by LC/ESI-MS analysis of produced water sample (SPT3). (A) Full scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

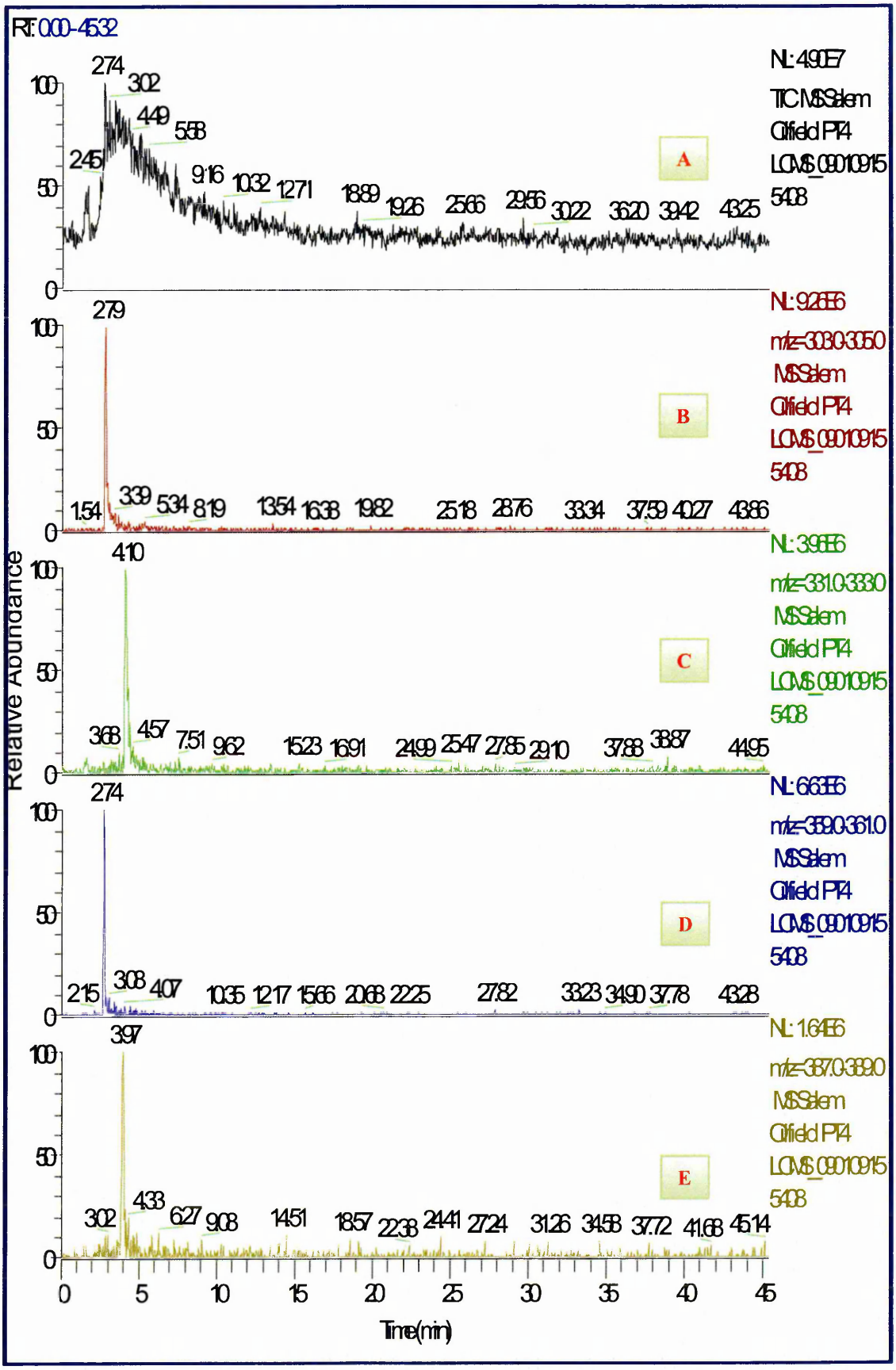


Figure 5-34: ESI extracted ion chromatograms of quaternary ammonium compounds obtained by LC/ESI-MS analysis of produced water sample (SPT4). (A) Full Scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

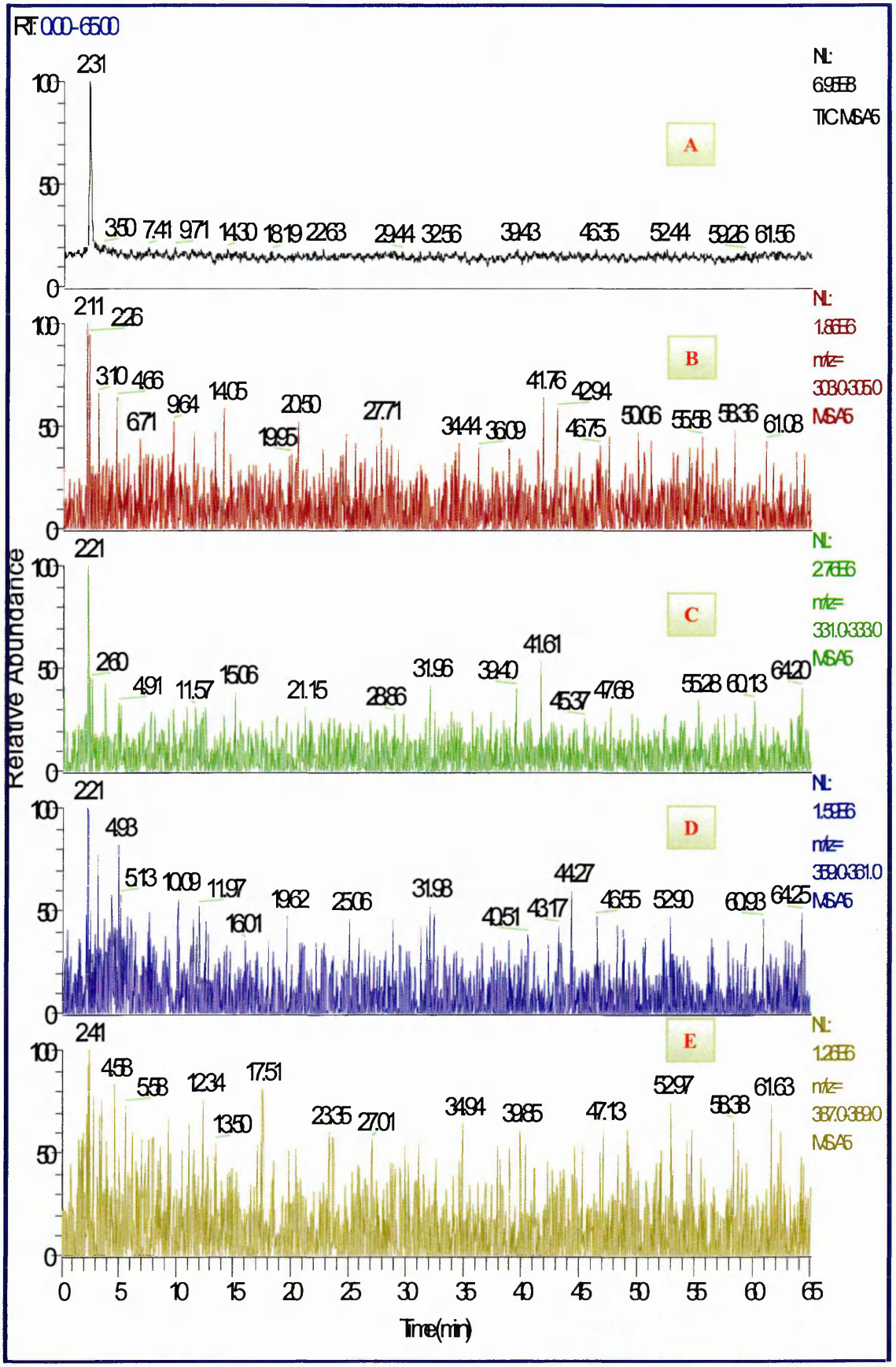


Figure 5-35 ESI extracted ion chromatograms of quaternary ammonium compounds obtained by LC/ESI-MS analysis of polluted soil sample (A5) Full scan 50-1850 (B) mass range 303-305 (C) mass range 331-333 (D) mass range 359-361 (E) mass range 387-389

5.5 Conclusions

PW is by far the largest contaminated stream resulting from oil and gas recovery operations. A number of oilfield chemicals (OFCs) are used during crude oil and natural gas recovery, production and separation to overcome operational problems and play a very important role in oil production. When PW is separated from crude oil and discharged into the disposal pit, some or all of these OFCs may be present in it. These may significantly affect the environment. This chapter presents a review of the nature and function of commercial OFCs that are commonly used in the Nasser oilfield and the qualitative characterization of this main group of oilfield chemicals in soil and PW samples collected from the Nasser oilfield, Libya. The most important conclusions which could be drawn from this chapter are:-

- DCM extraction and SPE techniques allowed us to clean and concentrate the compounds of interest.
- ESI-MS has been shown powerful technique for the identification of OFCs.
- SPE followed by ES-MS and HPLC coupled together has been proved very useful in the characterization of an important class of OFCs in soil and PW.
- LC coupled with ESI -MS method shows it is possible to separate OFCs peaks from other interfering peaks.
- Benzalkonium quaternary ammonium salts were successfully measured using SPE followed by ESI-MS and LC-ESI-MS in soil and PW samples collected from discharged pit at the Nasser oilfield, Libya.

Chapter Six
Enhanced biodegradation of petroleum hydrocarbons in crude oil contaminated soil

6.0 Introduction

As previously explained in Chapter 1, crude oil is a complex mixture of a wide range of hydrocarbons and non-hydrocarbon compounds of different properties. Crude oils contain many different hydrocarbon compounds that vary in appearance and composition from one oilfield to another (Agarry et al, 2010 and Salam et al., 2011). The hydrocarbons present have a varying number of carbon atoms, which can range from 1 to more than 100. Light oil has a greater proportion of the low molecular weight components while heavy oil has more of the high molecular weight components. The lower molecular weight components, particularly those with fewer than 15 carbon atoms, evaporate rapidly while the presence of higher molecular weight components tends to make the oil more viscous and less-volatile. The components of crude oil have been classified into four distinct compound types - saturated hydrocarbons, aromatics, resins and asphaltenes. In comparison to the saturated and aromatic fractions, the resin and asphaltenes contain non-hydrocarbon polar compounds. Resins and asphaltenes have very complex and mostly unknown carbon structure with addition of many nitrogen, sulfur and oxygen atoms (Nocentini et al., 2000, Harayama et al., 2004, Speight 2007 and Gojgic-Cvijovic et al., 2012). This chapter describes the bioremediation method of crude oil contaminated soil collected from the Nasser oilfield, Libya using biostumulation and bioaugmentation techniques.

Crude oil is a major source of energy worldwide and an important environmental pollutant and causes a risk to the environment and presents a major challenge for the environmental researcher to find methods to clean up oil contaminated soils or water. Pollution by crude oil and its derivative is a serious environmental problem in all parts of the world. Depending on the source of the pollution (spill, waste disposal accidents), it can cause danger to either onshore or offshore ecosystems (Khan et al, 2004, Verma et al., 2005, Schaefer et al., 2007 and Liu et al., 2009[2]). The light oils seem to cause the least problems at sea; it is the opposite situation on land. Light oil tends to penetrate the top soil rapidly thus seeping into the deeper ground and putting the ground water at risk. On the other hand, heavier oil slowly contaminates the soil as its higher viscosity makes penetration difficult. Depending on the viscosity, spilt oil will flow, more or less fast, to the lower part of the landscape. Apart from this, spreading will not take place so the evaporation of the lighter fractions of the oil will be limited (ITOPF 2004 and 2007).

The crude oil are extracted, produced, refined and handled on land every day and although improvements in careful handling, transportation have been made there is still the possibility that some may enter the environment via spill, leaking or the disposal pits

of waste petroleum and accidental during transport (Chaineau et al., 2005 and Malik et al., 2012). Industry and the public are now aware of the potential risk that complex chemical mixtures such as petroleum hydrocarbons (PH), polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), heavy metals and pesticides pose to human health and the environment (Khan et al., 2004).

6.1 Bioremediation as a treatment option

In response to a growing need to clean-up environmental contamination, various remediation technologies have been developed to treat soil, wastewater and groundwater contaminated by different pollutants. These include *in-situ* and *ex-situ* methods. The choice of the remediation method depends on the type, extent, concentration of the pollutant and the future land use. A combination of methods (biological, physical, and chemical technologies) may be used to decrease the contamination to a harmless and suitable level (Khan et al, 2004, Verma et al., 2005 and Malik et al., 2012).

The need to remediate contaminated sites is essential for protecting human health and environmental ecosystems. There is an increasing interest for government and industry in promoting environmental methods in the process of cleaning oil-polluted sites. Compared to physiochemical methods, bioremediation offers a very feasible alternative method for the treatment of oil in the environment; this technique is considered an effective technology for treatment of oil pollution when compared to physical and chemical methods (Xu et al., 2005, Salam et al., 2011 and Malik et al., 2012). Bioremediation is defined as the use of living organisms, to degrade environmental contaminants into harmless by-products. When the environment is contaminated with petroleum hydrocarbons the proportion of hydrocarbon degrading microorganisms increases rapidly (Atlas 1995, Riser-Roberts 1998, Xu et al., 2005, Das et al., 2011 and Malik et al., 2012). There are two major problems related to a hydrocarbon's chemical structure that can prevent its biological breakdown. First, the molecule may contain groups that cannot react with available enzymes. Second, the structure may determine the compound to be in a physical state where microbial degradation does not easily occur. Many microorganisms possess the enzymatic capability to degrade petroleum hydrocarbons. Some microorganisms degrade alkanes, alkene, others aromatics, and others both paraffinic and aromatic hydrocarbons. Typically, the larger and more complex the structure of a hydrocarbon the more resistant it is to biodegradation, i.e. it is oxidized slowly. Often the normal alkanes in the range C_{10} to C_{26} are viewed as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene

and xylene, which are among the most toxic compounds found in petroleum, are also very readily biodegraded by many microorganisms. Bioremediation is one of the most important methods for the remediation of contaminated soil with petroleum hydrocarbons, as it is environmental friendly and a relatively low cost effective (Atlas 1995, Riser-Roberts 1998, Xu et al., 2005, Das et al., 2011 and Malik et al., 2012).

Bioremediation is not a new concept. Microbiologists have studied the process since the 1940s. As reviewed by Atlas (1981); Claude E. ZoBell, in 1946 evaluated the effect of microorganisms on hydrocarbons. He recognized that those microorganisms widely distributed in environment have the ability to utilize hydrocarbons as the sole source of carbon and energy. ZoBell concludes that the microbial utilization of hydrocarbons depended upon the type, concentration of hydrocarbon and environmental conditions. However, bioremediation became known to a broader public in the U.S., only in the late 1980s as a technology for cleanup of shorelines contaminated with spilled oil. In the years since 1989, bioremediation has become a technology that is discussed, applied, and considered in many different circumstances (Hoff 1993).

Many published articles from the 1970s and earlier documented the process of microbial degradation of oil, both in the laboratory and in field trials. A number of scientific papers on this topic were published during the 1970s and 1980s, including several review papers covering mechanisms of biodegradation, and papers presenting results from controlled field experiments measuring degradation rates in various environments. Several studies following major oil spills measured oil degradation in the environment and confirmed previously published results from laboratory studies (Hoff 1993). During the 1980s numerous commercial products were developed for use as bioremediation agents. Some of these products primarily used nutrients, but the majorities were derived from the growing biotechnology industry and included microbes of various kinds. During 1989 and 1991 with bioremediation receiving wide attention and interest the US-Environmental Protection Agency (EPA) was a strong force for using bioremediation (Hoff 1993). In addition, A number of laboratory and field tests have established that bioremediation could be a cost-effective clean-up technology to treat oil polluted soils and sediments containing biodegradable hydrocarbons and indigenous specific microorganisms able to breakdown pollutants (Atlas 1981, Huesemann et al., 1993, Bossert et al., 1995 Wange et al., 1995[2] Jackson et al., 1996, Venosa et al., 1996, Salanitro et al., 1997, Nocentini et al., 2000, Gojgic-Cvijovic et al., 2012, and Boopathy et al., 2012).

The organisms that occur naturally in almost every soil (indigenous microbial populations) appear to be chief the agents involved in the metabolism of the pollutants in soil and water. Bacteria and fungi are responsible for most of the chemical transformations. Soil bacteria may be present in a dormant or slow growing sate, but when stimulated by favourable environmental conditions, they multiply rapidly and subsequently prosper in the new environment (Mohamed et al., 2005 and Mancera-Lopez et al., 2008). The main metabolic pathways for aerobic biodegradation of hydrocarbons have been elucidated, in which the initial steps in the biodegradation of hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygen. Hydrocarbons are subsequently converted to the non-toxic end products carbon dioxide and water, as well as cell biomass which can be safely disposed into the environment (Atlas 1995).

The principal bacteria and fungi genera responsible for oil degradation in both soils and aquatic environment have been identified as comprising mainly (*Pseudomonas*, *Acinetobacter*, *Achromobacter*, *Bacillus, Pseudomonas fluorescens*, *Marinobacter aquaolei VT-8* (known as *Pseudomonas nautical*), *Micrococcus*, *Nocardia*, *Flavobacterium*, *Rhodococcus*, *Trichoderma*, *Mycobacterium*, *Haemophilus*, *Arthrobacter*, *Aspergillus*, *Paenibacillus*, *Corynebacterium*, *Microbacterium*, *Ralstonia*, *Morteilla, Oleispiraantarctica RB-8^T and Ɣ-Proteobacterium*) (Atlas 1981, Leahy et al., 1990, Riser-Roberts et al., 1998, Song et al, 2006, Yakimov et al., 2007, Joo et al., 2008, Haritash et al., 2009, De Carvalho et al., 2011, Das et al., 2011, Onuoha et al, 2011 and Malik et al., 2012). These microorganisms modify their cell surface to increase its affinity for hydrophobic substrates and, thus facilitate their absorption (Cybulski et al., 2003 and Carvalho et al, 2005). Degradation in the contaminated soil and water may be affected by environmental constraints such as dissolved oxygen, pH, temperature, oxidation- reduction potential, availability of inorganic nutrient (nitrogen and phosphorus), salinity and the concentration and the nature of the organics. The number and the type of organisms present in the environment will also play an important role in the degradation process. Therefore, treatment generally consists of optimizing conditions of pH, temperature, soil moisture content, soil oxygen and nutrient concentration to simulate the growth of the organisms that will metabolize the contaminants present (Riser-Roberts 1998, Ran et al, 2005, Joo et al., 2008, Liu et al, 2010, Amezcua-Allieri et al., 2012 and Zhang et al., 2012). Optimum environmental conditions and nutrient application rates generally have to be established in laboratory scale studies and small field trials. Some hazardous compounds may be degraded more

readily under aerobic conditions (the degradation of compounds by microorganisms in the presence of oxygen) and some compounds under anaerobic conditions (the degradation of compounds by microorganisms in the absence of oxygen). Remediation might, therefore, consist of a combination of both aerobic and anaerobic treatment methods (Riser-Roberts 1998 and Xu et al., 2005, Tyagi et al., 2011 and Gojgic-Cvijovic et al., 2012). There are several different bioremediation techniques. The underlying idea is to accelerate the rates of natural hydrocarbon biodegradation by overcoming the rate-limiting factors. Biodegradation techniques are flexible and can be used at different stages of treatment or with different approaches. For instance, the indigenous microbial biomass is the key choice for an oil spill bioremediation using a biostimulation method in an open environment. This is generally achieved by adding nutrients or other substances to the soil to enhance natural attenuation to stimulate the growth of indigenous oil degrading microorganisms. Where the introduction of extra microorganisms into the soil this is called bioaugmentation. Biostimulation and bioaugmentation are widely known technologies to remediate hydrocarbon polluted sites. Bioremediation is one of the most important methods for the remediation of contaminates soil with petroleum hydrocarbons as it is environmentally friendly and a relatively low cost (Riser-Roberts 1998, Ran et al, 2005, Xu et al., 2005, Menendez-Vega et al., 2007, Liu et al, 2010, Tyagi et al., 2011, Amezcua-Allieri et al., 2012, Gojgic-Cvijovic et al., 2012, and Taccari et al., 2012).

Bioremediation techniques have been used for years to remediate hydrocarbon polluted sites. Usually the typical soils often contain the microorganisms required for degrading compounds formed in nature but bioaugmentation of the populations may speed up the rate of bioremediation (Kim et al., 2005 and Tyagi et al., 2011). During the first Gulf War crude oil spills were created across the desert in Kuwait and about 6 - 107 million barrels of oil were spread over 20 km^2 of soil and 320 oil lakes were made. The bioremediation method was the technology used to clean up these oil lakes at the studied site (Al-Saleh et al., 2005).

6.2 Microorganisms

In recent years, microbial ecologists have identified various microbial species that are effective degraders of hydrocarbons in natural environments. Many of these microbial consortia (i.e. mixture of strains or species working together) have been isolated from heavily contaminated areas. They were isolated according to their ability to metabolize various carbon sources, such as aliphatic and aromatic hydrocarbons and their

chlorinated derivatives. The driving force for petroleum biodegradation is the ability of microorganisms to utilize hydrocarbons to satisfy their cell growth and energy needs. A large number of studies report that low molecular weight alkanes are degraded most rapidly and that mixed cultures carry out more extensive biodegradation of petroleum than pure cultures (Trindade et al., 2005, Mohamed et al., 2005 and Mancera-Lopez et al., 2008).

In many ecosystems there is already an adequate indigenous microbial community capable of extensive oil biodegradation, provided that environmental conditions are favourable for oil-degrading metabolic activity. There are several advantages to relying on indigenous microorganisms rather than adding microorganisms to degrade hydrocarbons. First, natural populations have developed through many years. These microorganisms are adapted for survival and proliferation in that environment. Secondly, the ability to utilize hydrocarbons is distributed among a diverse microbial population. This population occurs in natural ecosystems and either independently or in combination metabolizes various hydrocarbons (Chaineau et al., 2005). Microorganisms are equipped with metabolic machinery to use petroleum products as a carbon and energy source. The metabolic pathways that hydrocarbon degrading heterotrophs use can be either aerobic (i.e. they utilize oxygen as the primary electron acceptor) or anaerobic (i.e. they utilize an alternative electron acceptor such as nitrate or sulphate). Aerobic degradation usually proceeds more rapidly and is considered to be more effective than anaerobic degradation (Atlas 1981).

6.2.1 Aerobic degradation

Aerobic bioremediation is the most important process in regard to petroleum hydrocarbons because this process is capable of destroying a large percentage of hydrocarbon contaminant mass under certain conditions. Destruction occurs as a result of bacteria oxidizing reduced materials such as hydrocarbons to obtain energy. Aerobic biodegradation of hydrocarbons and crude oil is a mature and well studied process. Organic constituents have a more rapid rate of biodegradation under aerobic conditions as compared to anaerobic conditions. Therefore, aerobic processes are considered to be more important for the bioremediation of petroleum (Atlas 1981). The hydrocarbons are broken down by a series of enzyme-mediated reactions. Oxygen serves as an external electron acceptor, while an organic component of the contaminating substance functions as the electron donor or energy source (Hamme et al., 2003).

6.2.2 Anaerobic degradation

Anaerobic degradation is the process of the degradation of compounds by microorganisms in the absence of oxygen. In this situation microorganisms use another species (commonly nitrate, sulphate or iron) rather than oxygen as the terminal electron acceptor (Chakraborty et al., 2004). By the late 1980s groups of microorganisms were found to degrade hydrocarbons under anaerobic conditions. Studies have confirmed that these microorganisms activate organic compounds by biochemical mechanisms that differ from those employed in aerobic hydrocarbon metabolism (Chakraborty et al., 2004). N-alkanes, branched alkanes, cycloalkanes, and some alkenes have been shown to be degraded under anaerobic conditions (Widdel et al, 2001 and Chakraborty et al., 2004). Several laboratory and field studies conducted on biodegradation had clearly demonstrated biodegradation of BTEX (benzene, toluene, ethylbenzene and xylene isomers) compounds. The biodegradation of these individual compounds depends on the terminal electron acceptor. Usually, toluene and m, p-xylenes degrade under anaerobic conditions (Chakraborty et al., 2004 and Schreiber et al., 2004).

Aerobic microorganisms degrade a larger range of hydrocarbon compounds than anaerobic. Aerobic bioremediation processes are very effective in treating hydrocarbon contamination; however they are often expensive because of the costs associated to the aeration methods. For this reason anaerobic biodegradation provides a cost-effective and advantageous *in-situ* bioremediation technology that can be used for the decontamination of soil, sediment, and ground water contaminated with petroleum hydrocarbons (Atlas 1981 and Johnson et al., 2003). Anaerobic treatment could be followed by aerobic treatment to complete the biodegradation of the partially degraded hydrocarbons compounds (Atlas 1981 and Johnson et al., 2003).

6.3 Scope of this chapter

This chapter will define the requirements and the optimum conditions for biodegradation of crude oil-contaminated soils from the Nasser Oilfield, Libya by: -

- Comparing the biodegradation efficiencies obtained by applying bioaugmentation and biostimulation techniques to crude oil contaminated soils from the Nasser Oilfield Libya.
- Determining the best strategies of biostimulation, bioaugmentation and moisture adjustment to be applied in the biological treatment of the contaminated soil used in this study.
- Assessing the best biological soil parameters to monitor TPH degradation in soil.

- ❖ Determining the degradation rates at different conditions and evaluate the optimal rate as follow:
    - Base conditions.
    - Temperature control.
    - pH control
    - Moisture control
    - Pollutant concentration (TPH) control.
    - Nutrient concentration control.
    - Salinity effect.
- ❖ Screening samples of oil polluted soil for TPH-degrading microorganisms.
- ❖ Characterizing the isolated microorganisms by their molecular biology.
- ❖ Identifying the strains that may be used for the bioremediation of TPH by using 16S rRNA gene sequencing.

6.4 Materials and methods

6.4.1 Chemicals

Components of NMS medium were supplied by Sigma, UK, peptone, yeast extract, dextrose, purified agar, peptone; sodium chloride, disodium phosphate and monopotassium phosphate were supplied by Sigma, UK. All solvents used were of HPLC grade and were obtained from Sigma, UK. Crude oil contaminated soil samples used in this investigation were taken from the disposal pit at the Nasser Oilfield Libya.

6.4.2 Study area

The study area is situated at the Nasser Oilfield, which is located in the North Central part of Libya as previously explained in Chapter 2 sections 2-2. Soil samples contaminated with crude oil used in this study were collected from different sampling point and depths (0-15 cm) around the Nasser Oilfield disposal pit as shown in Chapter 2, Figures 2-17 and 2-18. Development of a bioremediation protocol requires a thorough site characterization including site geology and the distribution of contaminates.

6.4.3 Equipment

The autoclave was purchased from Classic Prestige Medical (UK), the orbital incubator shaker was obtained from Gallenkamp, UK, the freeze dryer model Modulyod 230 was purchased from Thermo Electron Corporation, UK, the Heraeus fanned incubator was

supplied by Kindro Laboratory Product, Germany and the gas chromatography flame ionization detector (GC-FID) model 500 equipped with autosampler was purchased from Perkin Elmer Clarus, USA.

6.4.4 Composition of NMS media, buffered peptone water and nutrient agar used in this investigation

- NMS liquid medium

  NMS liquid medium contained (g/l): 10g/l KNO_3, 10g/l $MgSO_4.7H_2O$, 10g/l $CaCl_2.2H_2O$, 0.5g/l $NaMoO_4.2H_2O$, 3.8 g/l FeEDTA, 49.7g/l, Na_2HPO_4, 39g/l KH_2PO_4 and 1 ml trace element solution (pH 7.0). Trace elements solution contained (mg/l) 100mg $CuSO_4.5H_2O$, 500mg $FeSO_4.7H_2O$, 400mg $ZnSO_4$. $7H_2O$, 15mg H_3BO_3, 50mg $CoCl_3.6H_2O$, 250mg Na_2EDTA, 20mg $MnCl_2.4H_2O$ and 10mg $NiCl_2.6H_2O$. Then the medium was sterilized by autoclaving for 20 minutes at 121°C.

- NMS agar

  NMS agar (solid media) contained (g/l): 10 g/l KNO_3, 10 g/l $MgSO_4.7H_2O$, 10 g/l $CaCl_2.2H_2O$, 0.5 g/l $NaMoO_4.2H_2O$, 3.8 g/l FeEDTA, 49.7 g/l, Na_2HPO_4, 39 g/l KH_2PO_4 and 1ml trace element solution and 15g/l purified agar. Crude oil (5% w/v) was used as the source of carbon and energy. Then the medium was sterilized by autoclaving for 20 minutes at 121°C.

- Buffered peptone water contained: peptone 10 g/l, sodium chloride 5 g/l, disodium phosphate 3.5 g/l and monopotassium phosphate 1.5 g/l was used for serial dilution. Then the buffered peptone water was sterilized by autoclaving for 20 minutes at 121°C

- Nutrient agar (solid media) contained: peptone 5 g/l, yeast extract 2.5 g/l, dextrose 1.0 g/l and purified agar 15 g/l was prepared and sterilized by autoclaving for 20 minutes at 121°C and then were used for investigation.

6.5 Experimental design of laboratory bioremediation experiments

Surface contaminated soil samples (0–15 cm below surface) with different levels of total petroleum hydrocarbon and salinity from the Nasser Oilfield, Libya were used in this study. The experimental setup consists of 18 different treatment units as shown in Tables 6-1A, B and C).

Table 6-1A Experimental conditions and variants

Treatment Unit	TPH mg/kg	Salinity as NaCl (mg/kg)	Incubation Temp. (°C)	N:P ratio	NMS added	Culture
T2	72266.9	50514	25	n	n	n
T3	72266.9	50514	25	10:1	n	n
T4	72266.9	50514	25	5:1	n	n
T5	72266.9	50514	40	n	n	n
T6	72266.9	50514	40	10:1	n	n
T7	72266.9	50514	40	5:1	n	n
T8	72266.9	50514	25	n	y	n
T9	72266.9	50514	25	n	n	y
T10	72266.9	50514	25	5:1	n	y
T11	72266.9	50514	40	n	y	n
T12	72266.9	50514	40	n	n	y
T13	72266.9	50514	40	5:1	n	y
C1	64240.8	50514	25	n	y	n
C2	64240.8	50514	40	n	n	n

n= no, y= yes, N:P= Nitrogen: Phosphorous ratio

Table 6-1B Experimental conditions and variants

Treatment Unit	TPH mg/kg	Salinity as NaCl (mg/kg)	Incubation Temp. (°C)	N:P ratio	NMS added	Culture
T14	37963.1	18530	25	n	n	n
T15	37963.1	18530	25	10:1	n	n
T16	37963.1	18530	25	5:1	n	n
T17	37963.1	18530	40	n	n	n
T18	37963.1	18530	40	10:1	n	n
T19	37963.1	18530	40	5:1	n	n
T20	37963.1	18530	25	n	y	x
T21	37963.1	18530	25	n	n	y
T22	37963.1	18530	25	5:1	n	y
T23	37963.1	18530	40	n	y	n
T24	37963.1	18530	40	n	n	y
T25	37963.1	18530	40	5:1	n	y
C3	34877.2	18530	25	n	y	n
C4	34877.2	18530	40	n	n	n

n= no, y= yes, N:P= Nitrogen: Phosphorous ratio

Table 6-1C Experimental conditions and variants

Treatment Unit	TPH mg/kg	Salinity as NaCl (mg/kg)	Incubation Temp. (°C)	N:P ratio	NMS added	Culture
T26	8733.3	27956	25	n	n	n
T27	8733..3	27956	25	10:1	n	n
T28	8733.3	27956	25	5:1	n	n
T29	8733.3	27956	40	n	n	n
T30	8733.3	27956	40	10:1	n	n
T31	8733.3	27956	40	5:1	n	n
T32	8733.3	27956	25	n	y	n
T33	8733.3	27956	25	n	n	y
T34	8733.3	27956	25	5:1	n	y
T35	8733.3	27956	40	n	y	n
T36	8733.3	27956	40	n	n	y
T37	8733.3	27956	40	5:1	n	y
C5	8007.0	27956	25	n	n	n
C6	8007.0	27956	40	n	y	n

n= no, y= yes, N:P= Nitrogen: Phosphorous ratio

6.6 Biodegradation at lab scale experiments

The laboratory biodegradation experiments were carried out in 500 ml conical flasks [177 mm height and 50 mm diameter] containing 250 grams of contaminated soil collected from the study site at the Nasser Oilfield, the soil water content and pH were adjusted to 20-30 % of water holding capacity and 6.0 - 8.0 respectively. No further adjustments for other elements were made during the course of the study. The flasks were incubated for 240 days at 25°C and 40°C to simulate the temperature condition at the contaminated site. The contaminated soil samples namely A2, A4 and A6 taken from the Nasser Oilfield with TPH concentrations 8733.3, 37963.1 and 72266. 9 mg/kg soils respectively were used. The nutrient levels for the biodegradation of crude oil contaminated soil N:P was adjusted by adding quantities per kilogram of soil: NH_4Cl 3.05 g, K_2HPO_4 0.79 g, $(NH_4)_2SO_4$ 2.22 g and KH_2PO_4 0.61g to the flask to reach a N:P ratio of 10:1. There was a control without any nutrient addition in order to evaluate crude oil degradation rate in its own condition (natural attenuation). For each temperature of incubation (25°C and 40°C), eight specific conditions used for each sample are described as follows:-

- Control- Sterilised soil + sterilised water
- Contaminated soil + water added only
- Contaminated soil + 5:1 N:P
- Contaminated soil + 10:1 N:P
- Contaminated soil + 10 ml NMS
- Contaminated soil + 10 ml culture
- Contaminated soil + 10 ml culture + 5:1 N:P

The details of the bioremediation experimental condition are presented in Tables 6-1A, B and C. The treatment flasks were incubated in the incubator under aerobic conditions for a period of eight months as shown in Figures 6-1. During the experiment, soil samples were taken from the treatment flask and analysed periodically (0, 60, 150 and 240 days) for the determination of TPH, soil pH, soil moisture and microbial activities or availability. The specific conditions which are used for each set are described in Table 6-1A, B and C. The control soil samples (C1, C2, C3, C4, C5 and C6) were sterilised by autoclaving for 20 minutes at 121°C to avoid microbial activity. The water content was checked regularly by taken soil sample from treatment flask and analysed for water holding capacity which then adjusted with sterilized water to maintain to about 20-30 %. During the incubation time periodic renewal of the flasks was made by

opening and aerating them (i.e. removing the cotton and aluminium foil from the flasks) for few minutes to allow sufficient oxygen to enter the flask as shown in Figure 6-1. In those experiments, the microbial activity was monitored periodically.

Figure 6-1 Microcosm study

6.7 Enrichment and isolation of bacterial

The bacteria used in the bioaugmentation experiments were isolated by enrichment culture techniques from the soil obtained from the polluted site at the Nasser oilfield as follows: Ten grams of the polluted soil samples were incubated in 200 ml of NMS liquid medium containing 10 ml of crude oil (5 % w/v) as carbon source and incubated at 37°C on a rotary shaker (150 rpm) for 7 days. After that, 10 ml aliquots were transferred to 100 ml of fresh liquid NMS medium containing 5 % sterile crude oil and incubated at 37°C on rotary shaker (150 rpm) for another 7 days. This step was repeated two times to enrich for microbial strains that could degrade the oil. Pure cultures were obtained by spreading 200 µl of aliquots on the solid NMS plate and nutrient agar plates containing 1 % crude oil as energy and carbon source and incubated at 30°C up to 5 days. After the incubation period, the number of bacterial colonies on the plates was counted. The bacterial colonies obtained were further purified on NMS agar. All isolates were stored at −80°C in the liquid cultures containing 20 % glycerol (v/v).

6.8 Microbial monitoring method

The monitoring of microbial content was conducted using the serial dilution plate method. One gram of each soil sample was added to 9 ml of buffered peptone water from which a series of aqueous solutions representing tenfold dilution were prepared. The bacteria were enumerated on nutrient agar plates and total petroleum hydrocarbon (TPH) degraders were monitored by the serial dilution plate method. Each sample dilution was swirled for 30 second to extract adsorbed bacteria from soil grains. 0.1 ml dilutions were placed on three type of petri-dishes Nutrient Agar, Nutrient agar containing 1 % crude oil and NMS containing 1 % crude oil using spread plate technique and incubated at 30°C for up to 4 days. Since in the majority of cases it was not possible to discern single colonies, the dilutions upon which microbial growth was seen were recorded for each set of samples. In order to obtain single colonies of oil-degrading microorganisms, the bacterial growth from the NA plates above was then streaked on to nutrient agar containing 1 % crude oil and NMS containing 1 % crude oil to determine if bacterial colonies could be isolated capable of been hydrocarbon tolerant and hydrocarbon utilizing respectively.

6.9 Monitoring of soil parameters

Initially and on 60, 150 and 240 days soil samples were taken from each treatment flask and analysed for a range of parameters.

I. Soil pH

A pH probe was calibrated and used to measure pH of each soil samples. Four grams of soil samples were taken from each treatment flask were weighed out and equal amounts of deionised water (DW) were added and mixed and allowed to equilibrate for 15 minutes then the pH, electrode was dipped into a mixture of soil sample and deionised water, then the measured of pH was carried out using pH meter type Jenway, model 350.

II. Soil moisture content

About five grams of soil samples (weigh accurately to obtain the initial mass of sample, M1) were taken from each treatment flask and placed in a weighed crucible and dried at 105°C in an oven until a constant mass (M2) was reached. The percentage moisture was calculated from the difference according to the following equation:

$$\text{Water content (\%)} = [M1-M2/M1] \times 100$$

Where

M1 = Mass of soil before drying

M2 = Mass of soil after drying (at 105°C)

III. Hydrocarbons analysis (as total petroleum hydrocarbons (TPH)

As described in greater details above, crude oil contains a mixture of compounds which can be divided into four fractions: saturated, aromatics, resins and asphaltene. To determine the extent of biodegradation of crude oil contaminated soil the oil residues were extracted from contaminated soil using soxhlet extraction according to a modified version of EPA standard method SW-846 3550B (Soxhlet Extraction). Following the incubation time ten grams of soil sample were taken from the treatment flask and mixed with 10 g of sodium sulphate and extracted with DCM using the Soxhlet system, for 24 to 48 hours. The extract was then poured into a beaker and the solvent left to evaporate down to dryness at room temperature. The solvent was then exchanged to n-hexane. Sample extracts were processed through a clean-up and fractionation procedure designed to separate the extract into two fractions. The first fraction, total saturate petroleum hydrocarbons consisted of saturated hydrocarbons such as n-alkanes, branched alkanes and cycloalkanes. The second fraction, total aromatic petroleum hydrocarbons consisted of aromatic hydrocarbons which include the PAHs (Mills, et al., 1999). A standard open liquid chromatography column was prepared (A 20-30 cm glass column length and 10 to 15 mm bore) with glass wool (2-3 cm), 15 grams of activated silica gel (70- 230 mesh) was added with 6 gram of sodium sulphate into the top of the column as a drying agent to reduce some of the moisture present in the soil. The column was then eluted with a minimum of 25 ml of hexane to prime the column to ensure the column bed was wet, and that it had not dried out after elution (EPA method 1664 1999, EPA method 3630B 1996, EPA method 8015C 2000, Mills et al., 1999 and Peramaki et al., 2010). The sample extract redissolved in hexane (2 ml) was then poured onto the top of the column, using a small filter funnel. The column was eluted with a further 25 ml of hexane was used to collect the TPH. Then the saturated fractions were injected into gas chromatography type a Perkin Elmer Clarus Model 500 gas chromatograph equipped with Perkin Elmer auto-sampler and flame ionization detector. TPH were determined in the saturated fractions by the details methodology previously explained in Chapter 4 sections 4.2.3

6.10 Assessment of hydrocarbon biodegradation

After certain times (0, 60, 150 and 240 days), soil samples were taken from the treatment flasks and the hydrocarbons were extracted from the soil as explained above. The rate of biodegradation was determined from the decline in TPH hydrocarbon concentration at each time point. A decrease in concentration of hydrocarbon indicated the biodegradation activity occurring. Hydrocarbon distributions were determined by

GC-FID for the extracted hydrocarbon in order to identify the range of degradation. Biodegradation was expressed as a percent decrease compared to the petroleum hydrocarbon soil sample before incubation (day 0).

6.11 Molecular characterisation

In order to identify bacterial strains isolated from the hydrocarbon contaminated soil, DNA was extracted from soil and bacterial 16S rRNA genese were amplified by PCR using the primers 16S-F and 16S-R (Lane 1991) as detailed below.

6.11.1 Equipment used for molecular identification of bacteria

During the isolation and identification of bacteria from soil the following laboratory equipment and devices were used: Heraeus PICO 17 Centrifuge supplied from Thermo Scientific, Germany, Gel Electrophoresis (Mini protean®Tetrav cell model, manufacturer by BIORAD), UVP Bio Imaging System (EPI ChemoII Dark room, UK), QIAGEN Kit (QIAamp DNA mini kit) and Primus 96 plus Authorized Thermal cycler for PCR (MGW Biotech, UK), and QIAGEN Kit (MinElute PCR Purification Kit) for purification of PCR products obtained by Sample & Assay Technology.

6.11.2 DNA extraction from hydrocarbon-utilising bacteria

100 µl from enrichment culture of 1 gram soil in buffered peptone water was spread in NMS agar plates containing 1 % crude oil. Six isolated bacterial colonies different in shape and size were taken from NMS agar plates. First each bacterial colony was incubated in nutrient broth media over night at 37°C; DNA was extracted and performed using a DNA extraction QIAGEN Kit using the manufacturer's protocol as follows:

1ml of suspension of overnight grown bacteria was added into a 1.5 ml micro centrifuge tube, the liquid was centrifuged at 7500 rpm for 5 min then the supernatant was removed and 180µl of buffer ATL was added 20 µl proteinase K solution (20 mg/ml) was added and the content was mixed by vorteixing and incubated at 56°C for 1 hour. The tubes were mixed two to three times during incubation period to ensure efficient lysis. 200 µl of buffer AL was added and the contents were mixed by pulse vorteixing for 15 s and incubated at 70°C for 10 min. 200 µl of pure ethanol was added to the content and was mixed again by using pulse vorteixing for 15 s. The mixture was carefully transferred to the QIAamp spin column and centrifuged for 1 min at 8000 rpm, then the QIAamp spin column was placed in a 2 ml cleaned collection tube after that the QIAamp spin column was opened and 500 µl of AW1 buffer was added and was centrifuged at 8000 rpm for 1min. Again the QIAamp spin column was placed in a 2 ml cleaned collection tube after that it was opened and 500 µl of AW2 buffer was added and was centrifuged at full speed (14000 rpm) for 3min. The QIAamp spin column was

placed in a 1.5 ml cleaned Eppeindorf tube and was opened then 150 µl buffer AE was added and incubated for 1 min at room temperature in order to elute the DNA from the column, then the centrifugation was performed at 8000 rpm for 1 min. Another 150 µl buffer AE was added at the same previous Eppeindorf tube and incubated for 5 min at room temperature, then the tube was centrifuged at 8000 rpm for 1 min. An aliquot of 10 µl was run on a 0.8 % agarose gel electrophoresis with 2.5 µl ethidium bromide (5 mg/ml) and 1 kb DNA ladder, ready to use (Fermentas, 0.1 µg/µl) in order to assess the quality of the DNA yield.

6.11.3 Polymerase chain reaction amplification (PCR) of 16S rRNA

The DNA extracts were then amplified using primers 16S-F and 16S-R for the bacterial 16S rRNA gene as shown in Table 6-2. The reactions were carried out in a 50µl containing 1× PCR buffer, 2mM dNTPs mixture, 1µM each of primer, template DNA, and 1µl of 1U of *Taq* polymerase (Thermus aquaticus). The cycling programme for a PCR was carried out on a Thermo Scientific thermal cycler as shown in Table 6-3.

Table 6-2 16S rRNA gene-specific primers and the size of PCR product

Gene	Primer Sequence (5´- 3´)	Annealing temp. at (°C)	Expected amplicon size (bp)	Reference
16S rRNA	16S-F (AGAGTTTGATCMTGGCTCAG) 16S-R (TACGGYTACCTTGTTACGACTT)	50	1500	Lane 1991

Table 6-3 PCR thermal cycling conditions used

	Temp.	Time	Number of cycles
Initial Denaturation	95°C	5-10 min	1 cycle
Denaturation	95°C	30 sec	30 cycles
Annealing	50°C	30 sec	
Extension	68°C	1 min	
Final Extension	68°C	9 min	1 cycle
Hold at 10°C			

6.11.4 Purification of 16S PCR products and sequencing

The 16S rRNA gene PCR products were purified by MinElute PCR Purification Kit (QIAGEN) as described by the manufacture.

The following protocols for preparing good quality DNA templates for sequencing, the purification of the 16S rRNA gene PCR products was as follows:-

1 volume of PCR reaction was mixed with 5 volumes of buffer PB. The MinElute column was placed in a 2 ml collection tube and the sample was transferred completely to MinElute column and centrifuged for 1 min. The flow through was discarded, the MinElute column was placed back into the same tube. A 750 µl of buffer PE was added to a MinElute column and centrifuged for 1 min in order to wash the column. Again flow through was discarded, then the MinElute column was placed back in the same tube and centrifuged for an additional 1 min at maximum speed (13000 rpm). MinElute column was placed in a 1.5 ml cleaned microcentrifuge tube and 10 µl of buffer EB (10mM Tris-Cl, pH 8.5) was added in the centre of the membrane to elute DNA. Then the column was left to stand for 1 min, and then the sample was centrifuged for 1 min. A portion of 5 µl was run on a 0.8 % agarose gel electrophoresis with 2.5 µl ethidium bromide (5 mg/ml) in order to assess the quality of the DNA yield.

The purified DNA was sent for sequencing by using 16S-F primer at Eurofins MWG/ Operon Company, Germany, in order to identify the types of bacterial strain present in the tested soil samples.

6.12 Results and discussion

It is an enormous task to clean up the hydrocarbon contaminants in soil which have being introduced by the disposal of oily produced water from the Nasser Oilfield activity. The use of bioremediation, though a very cost effective and environmentally friendly technique, has been limited by various factors with oxygen being one of the most important factors. Oxygen, nutrients, temperature, pH, water content and salinity often limit the biodegradation of hydrocarbons. When such factors are not optimal or close to optimal for microbial growth or for their ability to utilize carbon as a source of energy, the natural biodegradation process of oil components is decreased. In the laboratory and field pilot tests generally the optimum environmental conditions such as pH, temperature and nutrient application rates should be controlled. Therefore, treatment generally consists of optimising such conditions as pH, temperature, soil moisture content, soil oxygen content and nutrient concentration (Abu et al., 2008 and Mercer et al., 2011). This is to stimulate growth of the organisms that will metabolise the particular contaminants present. For instance, it has reported by Leahy et al., 1990 and Abu et al., 2008 that the decrease in pH during bioremediation may be attributable to release of acidic intermediates. Several studies showed that the degradation of oil increased with increasing pH with optimum degradation occurred under slightly alkaline conditions (Abu et al., 2008 and Mercer et al., 2011).

The parameters monitored during this study included changes in pH, temperature, moisture content, microbial availability and total petroleum hydrocarbon removed. One of the initial goals of this study was to measure the reduction of total petroleum hydrocarbon in crude oil contaminated soil over 240 days of incubation. Initial characterization of the site revealed the total petroleum hydrocarbon concentration in three crude oil contaminated soil samples were used in this study namely A2, A4 and A6 with hydrocarbon concentrations (heavy, medium and low) containing high concentrations of about 7.2 %, medium of 3.8 % and low of 0.87 % TPH, respectively as shown in Tables 6-4A, B and C. The soils used in this investigation namely A2, A4, and A6 had a dark brown color and were oily and sticky. Table 3-7 presented in chapter 3 provides the chemical and physical characteristics of the crude oil contaminated soil from the Nasser Oilfield. The main goal of this study was to evaluate the biodegradation efficiency of crude oil contaminated soil from the Nasser Oilfield that is achieved when biostimulation and bioaugmentation methods are used at different treatment condition (i.e. temperature, TPH, and nutrients ratio).

6.12.1 Soil properties

The concentration of total petroleum hydrocarbons (TPH) measured by gas chromatography (GC-FID) in the soil samples taken from the pit of the Nasser oilfield used in the bioremediation experiments were 7.2 %, 3.8 % and 0.87 %. The previous assessment of the site had shown that the contaminated soil contains medium sand to very fine sand and had a dark brown colour and was oily and sticky. The water content of the contaminated soil ranged from 8.50 to 10.13 % and soil reaction pH of polluted soil was ranged between 7.1-7.9, this range is optimal for bioremediation, and thus no further amendment was needed to enhance the soil reaction. The level of heavy metals ranged from 1.67 to 3.99 mg/kg, < 0.03 to 12.39 mg/kg, 1.77 to 107.40 mg/kg and < 0.002 mg/ kg soil for Cu, Pb, Zn and Cd, respectively. The details of chemical and physical properties of soil used in this investigation are presented in chapter three (Table 3-4).

Table 6-4A, B and C show the variations in the total petroleum hydrocarbons concentrations as measured by GC-FID over times in all different treatments units and the percentage of decrease in TPH concentrations after 240 days of incubation. In general, results obtained showed a significant decrease in total petroleum hydrocarbon degradation rate in the first 60 days of incubation period losses in hydrocarbons were significantly higher compared to the respective sterile control. Indeed, sterilized soil control showed, on the 60^{th} day a decrease in the ranged of 4.75 to 8.67 %, the maximum reduction in control soil from all concentrations used ranged from 10.39 % to 17.72 % after 240 days of incubation as shown in Tables 6-4A, B and C and Figures 6-2, 6-3 and 6-4. As reported by Huesemann 1995, rates of hydrocarbon removal are greatest in the first 10-20 weeks in all treatment conditions. The decrease in TPH concentration in heavy, medium and low crude oil contaminated soils was 73.18 % to 85.32 %, 91.17 % to 98.12 %, and 91.85 % to 99.10 % respectively of the initial TPH levels after 240 days. The maximum reduction in the TPH concentrations from soil in all microcosms was observed in treated soil T28 (amended with 5:1N:P at 25°C), in which 99.10 % of TPH was removed after 240 days of incubation. While in the sterile soil control (C1, 2, 3, 4, 5 and 6) and during the whole experimental period (240 days), the showed a considerably lower decrease (up to 17.72 %) of contaminant level than the biologically active soil sample (Tables 6-4A, B and C).

6.12.2 Degradation of petroleum hydrocarbon from crude oil contaminated soil

Degradation of total petroleum hydrocarbon (TPH) was determined by GC-FID over a period of 240 days. Total peak area for the extracted hydrocarbon from soil were

measured and plotted via the time period of experiment. The bioremediation of crude oil contaminated soil were applied using biostimulation and bioaugmentation.

6.12.2.1 Biostimulation

Bioremediation treatment by adding inorganic nutrients significantly enhanced crude oil in soil degradation rate by the indigenous soil microorganisms compared to natural attenuation (non-fertilized). During the experiment, the total petroleum hydrocarbons (TPH) in polluted soil samples were measured at 0, 60, 150 and 240 days and the biodegradation level on day X was the difference in the TPH values between the day 0 and X then divided by the value of the day 0 and expressed as percentage.

Figures 6-2, 6-3 and 6-4 shows the decrease in total petroleum hydrocarbon from three concentrations of crude oil contaminated soil samples (i.e. high, medium and low TPH concentrations) as function of time using biostimulation methods in which two temperature (25°C and 40°C) and two ratios of inorganic fertilized per kilogram of soil 5:1 N:P (i.e. 1.53 g of NH_4Cl, 0.40 g of K_2HPO_4, 1.11 g of $(NH_4)_2SO_4$ and 0.31g of KH_2PO_4) and 10:1 N:P ratio (i.e 3.05 g of NH_4Cl, 0.79 g of K_2HPO_4, 2.22 g of $(NH_4)_2SO_4$ and 0.61g of KH_2PO_4) were used. A substantial decrease was observed in total petroleum hydrocarbon concentrations in all systems during the first 60 days of study which gave a percentage loss ranging between 43.24 % and 99.10 % depending upon treatment conditions. The highest degradation rate was achieved at 25°C in sample T28 (low TPH content) amended with 5:1 ratio of N:P, where approximately 99.10 % of the total petroleum hydrocarbons were removed after 240 days. The initial TPH concentrations level, 8733.32 mg/kg soil was reduced to 78.28 mg/kg soil. In sterile controls the maximum reduction into the petroleum hydrocarbons only 17.72 % of the initial hydrocarbons were eliminated and it is clear that microorganism found in soil were still capable of degrading hydrocarbon at high temperature (40°C). A lower degradation rate was achieved at 25°C in sample T2 (high TPH content) amended with water only, where approximately 73.18 % of the total petroleum hydrocarbons were removed after 240 days, the initial TPH concentrations level, 72266.89 mg/kg soil was reduced to 19384.90 mg/kg soil after 240 days of treatment. Data shows that the petroleum degradation rate decreased with increasing time. In sterile controls the reduction in to the petroleum hydrocarbons ranged from 4.7 % to 17.72 % of the initial hydrocarbons hence it is clear that microorganism found in soil was still capable of degrading hydrocarbon at low and high temperature.

Table 6-4A Total petroleum hydrocarbon (TPH in mg/kg) removed in soil over time and the percentage of reduction in treatment unit

Incubation time (Day) / Microcosm ID	0 day	60 days	150 days	240 days	% Decrease
T2	72266.89	41020.53	23819.10	19384.90	73.18
T3	72266.89	31514.63	19872.61	15911.19	77.98
T4	72266.89	30096.41	17547.70	14508.31	79.92
T5	72266.89	33083.30	19918.43	18462.68	74.45
T6	72266.89	31768.91	14469.97	12345.14	82.92
T7	72266.89	32046.18	14045.37	10607.05	85.32
T8	72266.89	33673.23	21319.35	18365.57	74.59
T9	72266.89	32046.18	24153.61	18163.17	74.87
T10	72266.89	32250.41	21806.70	14247.37	80.29
T11	72266.89	33892.78	24123.67	18497.62	74.40
T12	72266.89	26218.57	22993.17	13327.34	81.56
T13	72266.89	27179.49	22164.60	13125.47	81.84
C1	64240.80	59179.53	57240.53	54114.95	15.76
C2	64240.80	58545.69	53734.23	52858.72	17.72

Table 6-4B Total petroleum hydrocarbon (TPH in mg/kg) removed in soil over time and the percentage of reduction in treatment unit

Incubation time (Day) / Microcosm ID	0 day	60 days	150 days	240 days	% Decrease
T14	37963.13	13528.21	2854.70	2513.96	93.38
T15	37963.13	9867.20	1816.43	952.22	97.49
T16	37963.13	8398.97	2680.53	1692.70	95.54
T17	37963.13	9898.29	6136.62	2299.99	93.94
T18	37963.13	7417.13	1551.07	1269.59	96.59
T19	37963.13	6640.16	1498.97	713.61	98.12
T20	37963.13	6236.73	4993.69	3351.61	91.17
T21	37963.13	5537.03	4162.86	2125.33	94.40
T22	37963.13	5950.22	4293.52	3160.80	91.67
T23	37963.13	5090.47	3601.95	3261.41	91.41
T24	37963.13	8443.79	6190.99	2806.65	92.61
T25	37963.13	6709.32	4202.68	1855.78	95.11
C3	34877.24	33170.35	31954.90	31252.52	10.39
C4	34877.24	32592.05	30346.19	29269.27	16.10

Table 6-4C Total petroleum hydrocarbon (TPH in mg/kg) removed in soil over time and the percentage of reduction in treatment unit

Incubation time (Day) / Microcosm ID	0 day	60 days	150 days	240 days	% Decrease
T26	8733.32	3046.70	494.84	418.52	95.21
T27	8733.32	2371.93	808.68	476.73	94.54
T28	8733.32	2506.59	646.71	78.29	99.10
T29	8733.32	3146.23	629.24	430.94	95.07
T30	8733.32	1973.20	598.03	395.62	95.47
T31	8733.32	2106.91	460.16	240.19	97.25
T32	8733.32	2246.33	814.63	652.41	92.53
T33	8733.32	2214.20	880.76	646.31	92.60
T34	8733.32	1809.92	925.25	279.66	96.80
T35	8733.32	1881.77	1009.73	710.99	91.86
T36	8733.32	1819.99	1030.57	623.42	92.86
T37	8733.32	1892.98	898.64	362.59	95.85
C5	8007.02	7627.03	7539.02	7134.98	10.90
C6	8007.02	7477.88	7050.65	6958.96	13.09

Figure 6-2 shows the reduction of total petroleum hydrocarbon for high crude oil contaminated soil (72266.9 mg/kg). Data show a higher hydrocarbon degradation rate during the first 60 days of the incubation period in all systems compared to the following 180 days. The concentration of hydrocarbon decreased from 72266.89 mg/kg to 19384.90 mg/kg soil at 25°C and from 72266.89 mg/kg soil to 18462.68 mg/kg soil at 40°C of treatments T2 (amended with water only) and T5 (amended with 5:1 N:P ratio) respectively after 240 days of experiment period. The addition of fertilizer (5:1 N:P (i.e. 1.53 g of NH_4Cl, 0.40 g of K_2HPO_4, 1.11 g of $(NH_4)_2SO_4$ and 0.31g of KH_2PO_4) and 10:1 N:P ratio (i.e 3.05 g of NH_4Cl, 0.79 g of K_2HPO_4, 2.22 g of $(NH_4)_2SO_4$ and 0.61g of KH_2PO_4) per kg of soil) only slightly increased in the degradation of hydrocarbon after 240 days; little difference was observed between the effect of the fertilizers with the two N:P ratios. The biodegradation of hydrocarbons of soil highly polluted with crude oil followed the general decrease of total petroleum hydrocarbon with maximum degradation of 85.32 % for sample T7 amended with 5:1 N:P at 40°C after 240 days, where, the initial TPH concentrations level, 72266.89 mg/kg soil was reduced to 10607.05 mg/kg soil after 240 days of treatment, whereas, only 17.72 % of the initial hydrocarbons were eliminated in sterile control samples (C2) amended with water only at 40°C as shown in Figure 6-2 and Table 6-4A, thus it is clear that microorganism found in soil were capable of degrading hydrocarbon at 25°C and 40°C. Temperature is an important factor for controlling microbiological activity and hydrocarbon decomposition (Liu et al., 2009). According to Mercer et al., 2011, the hydrocarbon degradation in soil can occur at a wide range of temperatures and the most rapid degradation occurred between 30°C to 40°C. In addition it has been reported that evaporation and volatilization occur primarily during the first few days of an oil spill (Venosa 1996, and Abu 2008).

Figure 6-3 shows the decrease in total petroleum hydrocarbon for medium crude oil contaminated soil (37963.1 mg/kg) which ranged from 64.37% to 98.12% depending upon treatment conditions. Highest TPH removal was observed in fertilized soil (5:1 N:P ratio) at 40°C (treatment T19) after 240 days, in which 98.12 % of the hydrocarbon were removed, whereas, only 16 % of the initial hydrocarbons was eliminated in sterile control samples (C4) amended with water only at 40°C. The initial TPH concentrations level, 37963.13 mg/kg, soil was reduced to 713.61 mg/kg soil. Addition of nutrient (N and P) to enhance biodegradation of petroleum hydrocarbon has been studied by several researchers. Atlas 1995 suggested that the addition of nutrient (slow release) has been shown to increase rates of petroleum degradation. Lee et al., 2007 has examined the

effect of the addition of nutrient to soil contaminated with waste lubricants under different nutrient conditions for over a 105 days testing period. They conclude that bioremediation using biostimulation is a viable choice for the remediation of soil contaminated with waste lubricants. They found that when the nutrients are added to the polluted soil stimulation occurs in fertilized soil for hydrocarbon degradation activity compared to non-fertilized soil.

Figure 6-4 shows the decrease in total petroleum hydrocarbon for soil contaminated with low levels of crude oil (8733.3 mg/kg) in which the highest degradation was achieved at 25°C; where approximately 99.10 % of the total petroleum hydrocarbons was removed after 240 days, the initial TPH concentrations level, 8733.32 mg/kg, was reduced to 78.29 mg/kg, in fertilized soil (5:1 N:P ratio). A lower percentage (%) of reduction of hydrocarbon was observed in non fertilized soil of treatment code T2 (amended with water only) at a temperature of 25°C in which 93.38% of hydrocarbons were removed, whereas, only 13.1 % of the initial hydrocarbons was eliminated in sterile control samples (C6) amended with NMS only at 40°C as shown in Figure 6-4 and Table 6-2C. An example of the percentage (%) oil degradation of the total petroleum hydrocarbon during the biostimulation treatment of a medium crude oil contaminated soil for two temperatures at soil salinity of 18350 mg/kg NaCl is shown in Figure 6-5. These results of hydrocarbon biodegradation at the above temperatures suggest that there are little differences in the reduction of the total petroleum hydrocarbons from polluted soil.

In general, biodegradation rate increased rapidly during the first 60 days of incubation in all treatment flasks and this may be due increased in hydrocarbon degrading bacterial populations, which corresponds with the rapid depletion of hydrocarbons during these periods. However, the absence or presence of nutrients did not influence significantly differences in the biodegradation rate.

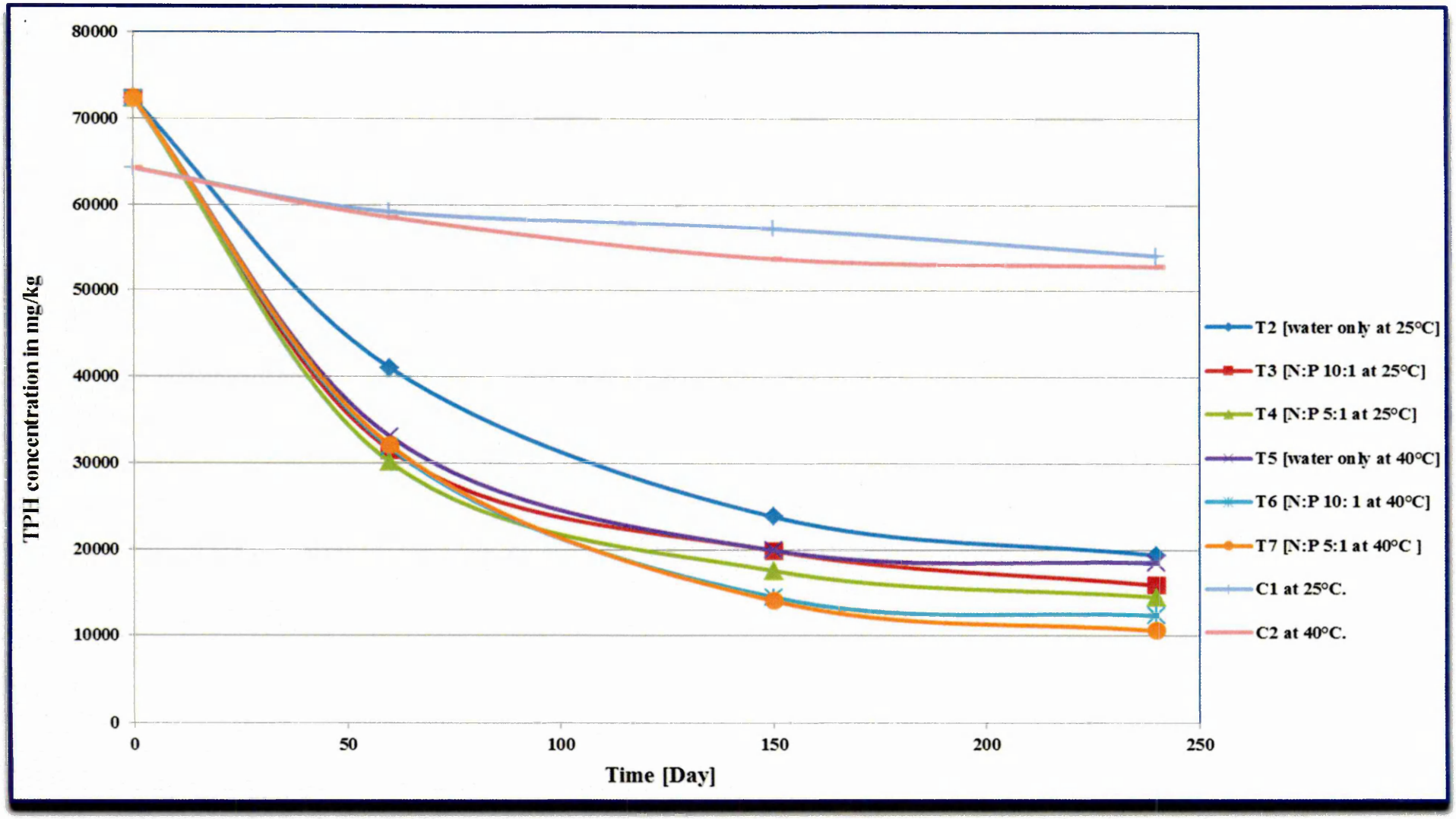


Figure 6-2 Removal of TPH from highly polluted soil for different treatments during biostimulation at 25 and 40°C

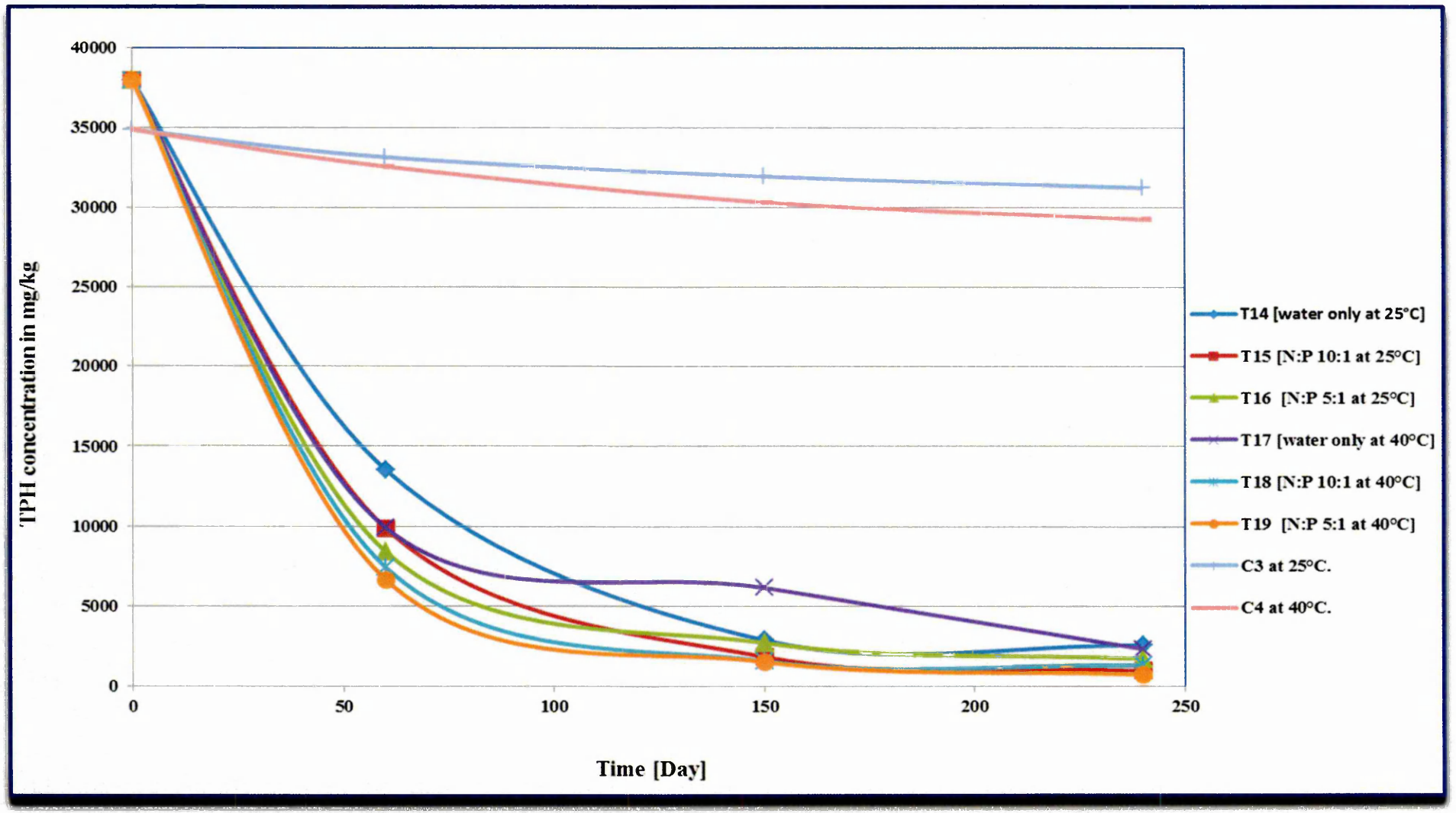


Figure 6-3 Removal of TPH from medium polluted soil for different treatments during biostimulation at 25 and 40°C

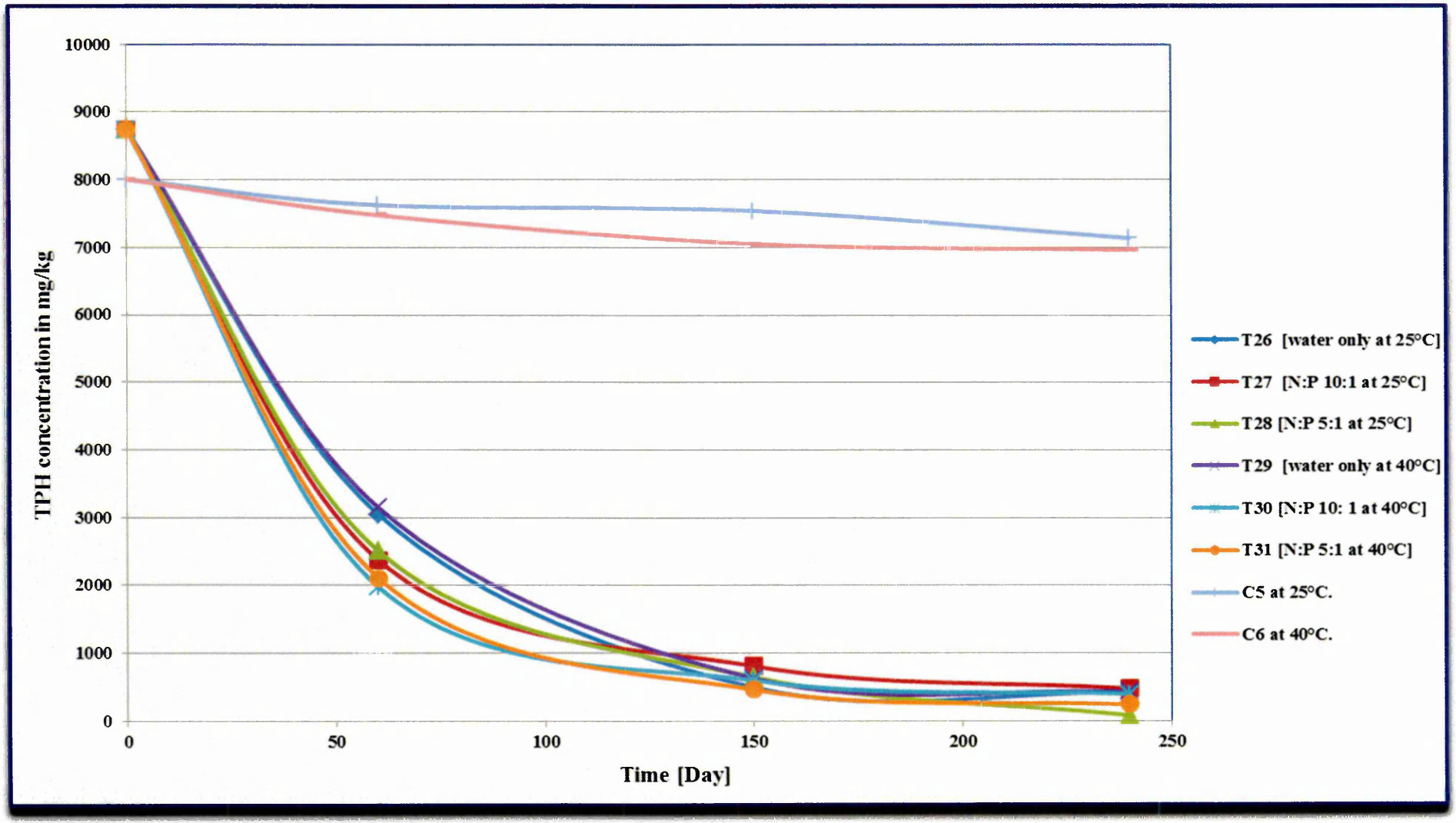


Figure 6-4 Removal of TPH from low polluted soil for different treatments during biostimulation at 25 and 40°C

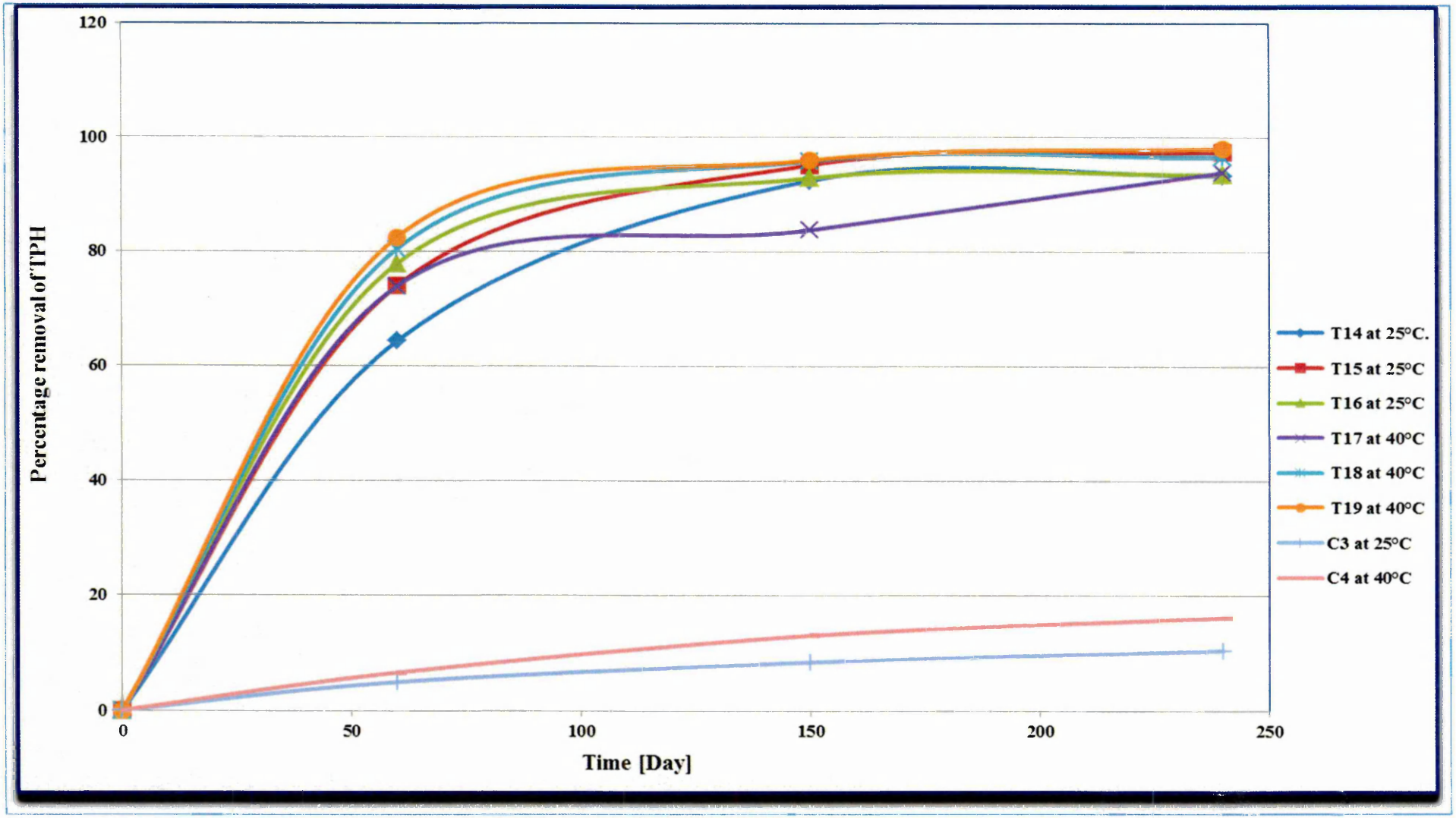


Figure 6-5 Percentage removal of TPH from soil during biostimulation constructed using data from Figure 6-3

6.12.2.2 Bioaugmentation

Bioremediation generally involves the use of hydrocarbon degrading microorganism as inocula. In this study the initial microbial consortium was isolated from the soil contaminated with crude oil of the study site. The microbial cultivation and bioaugmentation was carried out in nitrate mineral salt medium (NMS) in a 500 ml Erlenmeyer flask. Bioaugmentation was continued in the Erlenmeyer flask in the same nitrate mineral salt medium (NMS) with the addition a 5ml of microbial inoculum to the contaminated soil once during the 240 days of incubation. Therefore, an enrichment system for the high activity indigenous microbe was established and then these isolated microbes were introduced to the contaminated soil.

The efficiency of removal of total petroleum hydrocarbon from oily soil at 0, 60, 150 and 240 days for all oily soils used and all different treatments are shown in Figures 6-6, 6-7 and 6-8. Significant decreases in removal of total petroleum hydrocarbons were observed in all treatments flasks compared with the sterile control samples. However, the efficiency of hydrocarbons removal in all treated plots was higher than the control at the end of the experiment (240 days).

The degradation of total petroleum hydrocarbon in crude oil contaminated soil using bioaugmention treatment containing additional hydrocarbon degrading microbes ranged from 74.40 % to 96.80 % of hydrocarbon removed after 240 days of treatments, whereas, for the sterile control samples the total petroleum hydrocarbon removed ranged from 10.39 % to 17.72 %. The concentrations of total petroleum hydrocarbon decreased in all treated soils over time, up to 60 days. From day 60 to day 240, the biodegradation of hydrocarbons slowed down and little difference was observed in all treatment units as shown in Figures 6-6, 6-7 and 6-8 and Tables 6-4A, B and C.

Figure 6-6 shows the total petroleum hydrocarbon removal efficiency for high crude oil contaminated soil (72266.89 mg/kg). It is noticeable that the highest and fastest reductions of TPH occurred in the first 60 days of treatment for all treatment condition. In treatments unit T8 (at 25°C) and T11(at 40°C) which contain the addition of sterilized NMS only, the concentration of the hydrocarbon decreased after 240 days of treatments from 72266.89 mg/kg soil to 18365.57 mg/kg soil at 25°C and from 72266.89 mg/kg soil to 18497.62 mg/kg at 40°C for T8 and T11 respectively. The concentration of hydrocarbon decreased from 72266.89 mg/kg to 18163.17 mg/kg soil at 25°C and from 72266.89 mg/kg soil to 13327.34 mg/kg soil at 40°C for treatment unit which were amended with a 5 ml culture for soil samples T9 and T12 respectively. The addition of small amount of fertilizer 5:1 N:P (i.e. 1.53 g of NH_4Cl, 0.40 g of K_2HPO_4, 1.11 g of

$(NH_4)_2SO_4$ and 0.31g of KH_2PO_4 per kg of soil) and 10:1 N:P ratio (i.e 3.05 g of NH_4Cl, 0.79 g of K_2HPO_4, 2.22 g of $(NH_4)_2SO_4$ and 0.61g of KH_2PO_4 per kg of soil) pulse 5 ml culture to the treatments unit T10 (25°C) and T13(40°C) yielded little increase in the degradation of hydrocarbon after 240 days in comparison to the other treatments in which the initial concentration of hydrocarbon (i.e. 77266.89 mg/kg soil) was reduced to 14247.37 at 25°C and to 13125.47 for samples T10 (25°C) and T13 (40°C) respectively. The data shows that there were no large differences between the any of the treatments conditions used. The high degradation rate of hydrocarbons in soil highly polluted with crude oil was observed for treatment unit T13 (40°C) which was added the culture and N:P 5:1 ratio was observed. The highest degradation rate was observed for sample contain high TPH of sample T13 (amended with culture +5:1 N:P at 40°C) in which 81.84 % of hydrocarbons were removed after 240 days of treatment period, whereas, only 17.72 % of the initial hydrocarbon was eliminated in sterile control samples (C2, amended with sterile NMS only) as shown in Figure 6-6 and Table 6-4A. An example of the percentage (%) decrease of the total petroleum hydrocarbons during the bioaugmentation treatment of high crude oil contaminated soil (72266.89 mg/kg) for two temperatures at a soil salinity of 50514 mg/kg NaCl. The highest reduction was achieved in treatment unit T13 (amended with culture plus 5:1 N:P ratio at 40°C), in which 81.84 % of hydrocarbons were removed after 240 days of treatment. A lower percentage (%) of decrease of hydrocarbon was observed in treatment unit T11 (amended with NMS only at 25°C), in which 74.40 % of hydrocarbons were removed after 240 days of incubation.

Figure 6-7 shows the total petroleum hydrocarbon removal efficiency for medium crude oil contaminated soil (i.e. 37963.1 mg/kg). The highest degradation rate in hydrocarbons which occurred in the first 60 days were about 91.17 %, 94.40 %, 91.67 %, 91.41 %, 92.61 % and 95.11 % for treatments unit T20 (NMS only at 25°C), T21 (Culture at 25°C), T22 (Culture + 5:1 N:P at 25°C), T23 (NMS only at 40°C), T24 (Culture at 40°C) and T25 (Culture + 5:1 N:P at 40°C) respectively. The initial soil concentrations of hydrocarbon for treatments unit T20, T21 and T22 was 37963.13 mg/kg soil at 25°C which was reduced to 3351.61 mg/kg soil, 2125.33 mg/kg soil and 3160.80 mg/kg soil, respectively. A high percentage removal at 25°C was observed for treatment unit T21 in which 94.40 % of hydrocarbons was removed by the addition of a small amount of culture. The initial soil concentrations of hydrocarbon for treatments unit T23, T24 and T25 was 37963.13 mg/kg soil at 40°C which reduced to 3261.41

mg/kg soil, 2806.65 mg/kg soil and 1855.78 mg/kg soil, respectively. The high percentage reduction at 40°C was observed for treatment unit T25 in which 95.11 % of hydrocarbons was removed by the addition of a small amount of culture. In control sample (C4) the maximum hydrocarbon removed was observed at 40°C in which 16.1 % of hydrocarbon was removed. The initial concentration of hydrocarbon in the control sample (C4) was 34877.24 mg/kg soil and reduced to 29269.27 mg/kg soil as shown in Figure 6-7 and Table 6-4B.

Figure 6-8 shows the total petroleum hydrocarbon removal efficiency for low crude oil contaminated soil (8733.3 mg/kg). The results shows that the degradation of total petroleum hydrocarbons utilized in treatment units T32 (NMS only at 25°C), T33 (Culture at 25°C), T34 (Culture + 5:1 N:P at 25°C), T35 (NMS only at 40°C), T36 (Culture at 40°C) and T37 (Culture + 5:1 N:P at 40°C) were 92.53 %, 92.60 %, 96.80 %, 91.86 %, 92.86 % and 95.85 % respectively over the first 60 days of the time course of experiment. The results shows that the treatment unit involved the NMS only addition led to have the same decrease in TPH concentrations with the treatment unit with the addition of culture at both temperatures 25°C and 40°C after 240 days of treatments period. The initial soil concentrations of hydrocarbon for treatment units T32, T33, T35 and T36 was 8733.32 mg/kg soil and were reduced to 652.41 mg/kg soil, 646.31 mg/kg soil, 711.00 mg/kg soil and 623.42 mg/kg soil respectively. For the treatments unit T34 (Culture + 5:1 N:P at 25°C) and T37 (Culture + 5:1 N:P at 40°C) the data shows that there is litter difference TPH reduction with compared with other treatment condition. The initial TPH concentration for T34 and T37 was 8733.32 mg/kg soil and the concentrations was reduced to 279.66 mg/kg soil and 362.59 mg/kg soil respectively. The high percentage rate was observed at 25°C in treatment unit T34 in which 96.80 % of hydrocarbons were removed by the addition of a small amount of culture and 5:1 ratio of N:P. In the sterile control sample C6 (NMS only added) the maximum hydrocarbon removed was observed at 40°C which showed 13.10 % of hydrocarbon was removed. The initial concentration of hydrocarbon in the control sample was 8007.02 mg/kg soil and was reduced to 6958.96 mg/kg soil as shown in Figure 6-8 and Table 6-4C.

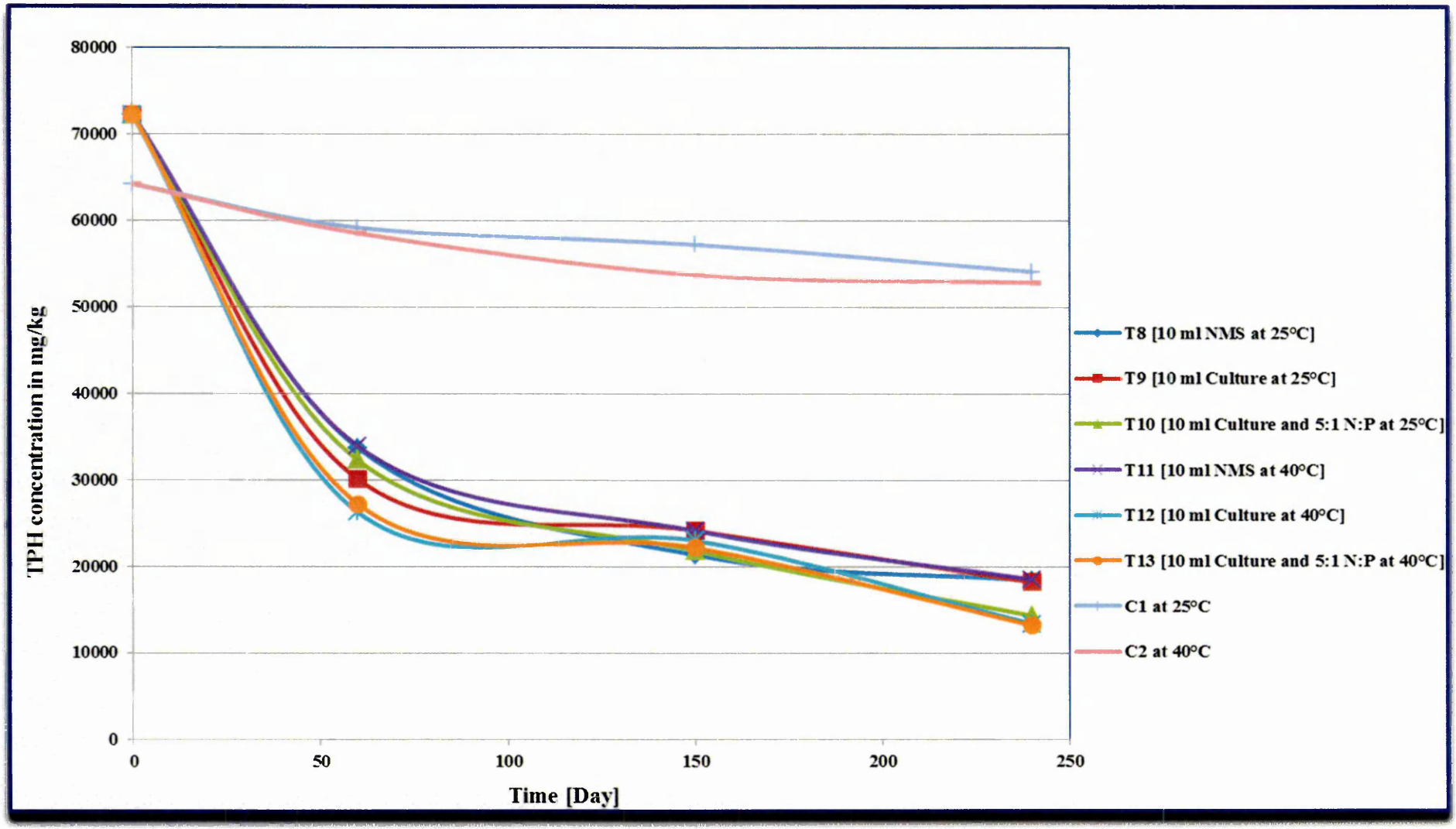


Figure 6-6 Removal of TPH from highly polluted soil for different treatments during bioaugmentation at 25 and 40°C

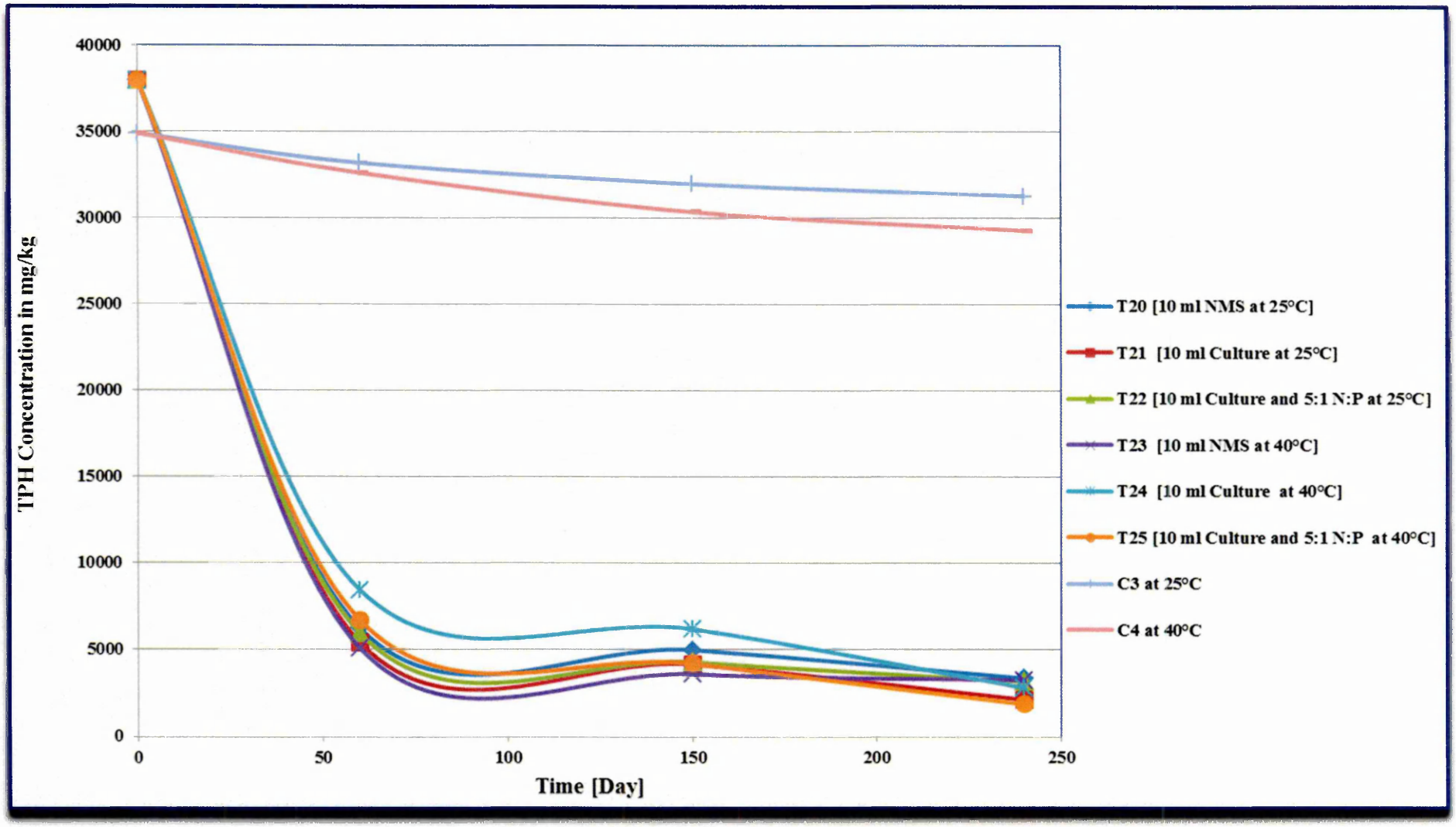


Figure 6-7 Removal of TPH from medium polluted soil for different treatments during bioaugmentation at 25 and 40°C

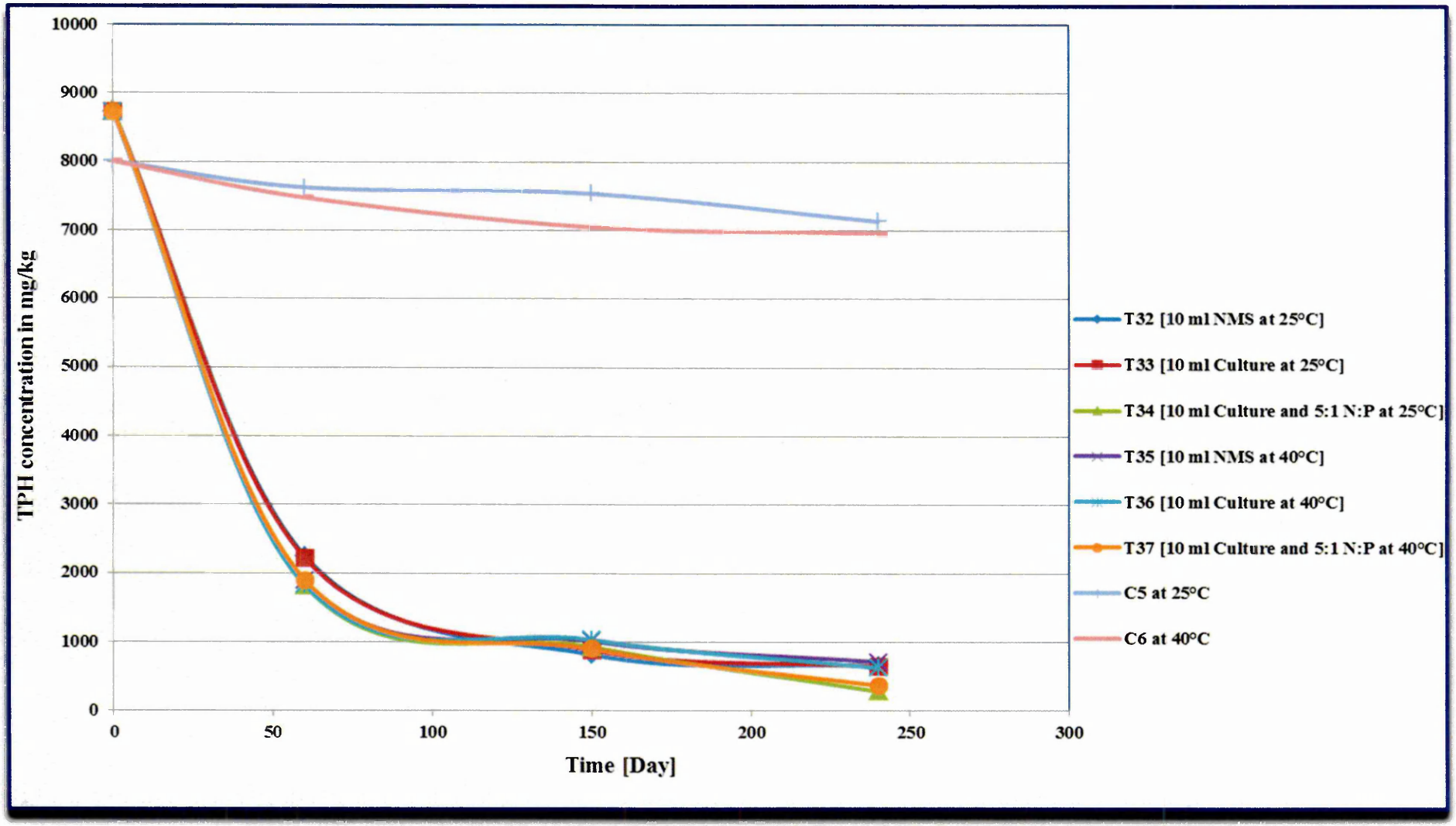


Figure 6-8 Removal of TPH from low polluted soil for different treatments during bioaugmentation at 25 and 40°C

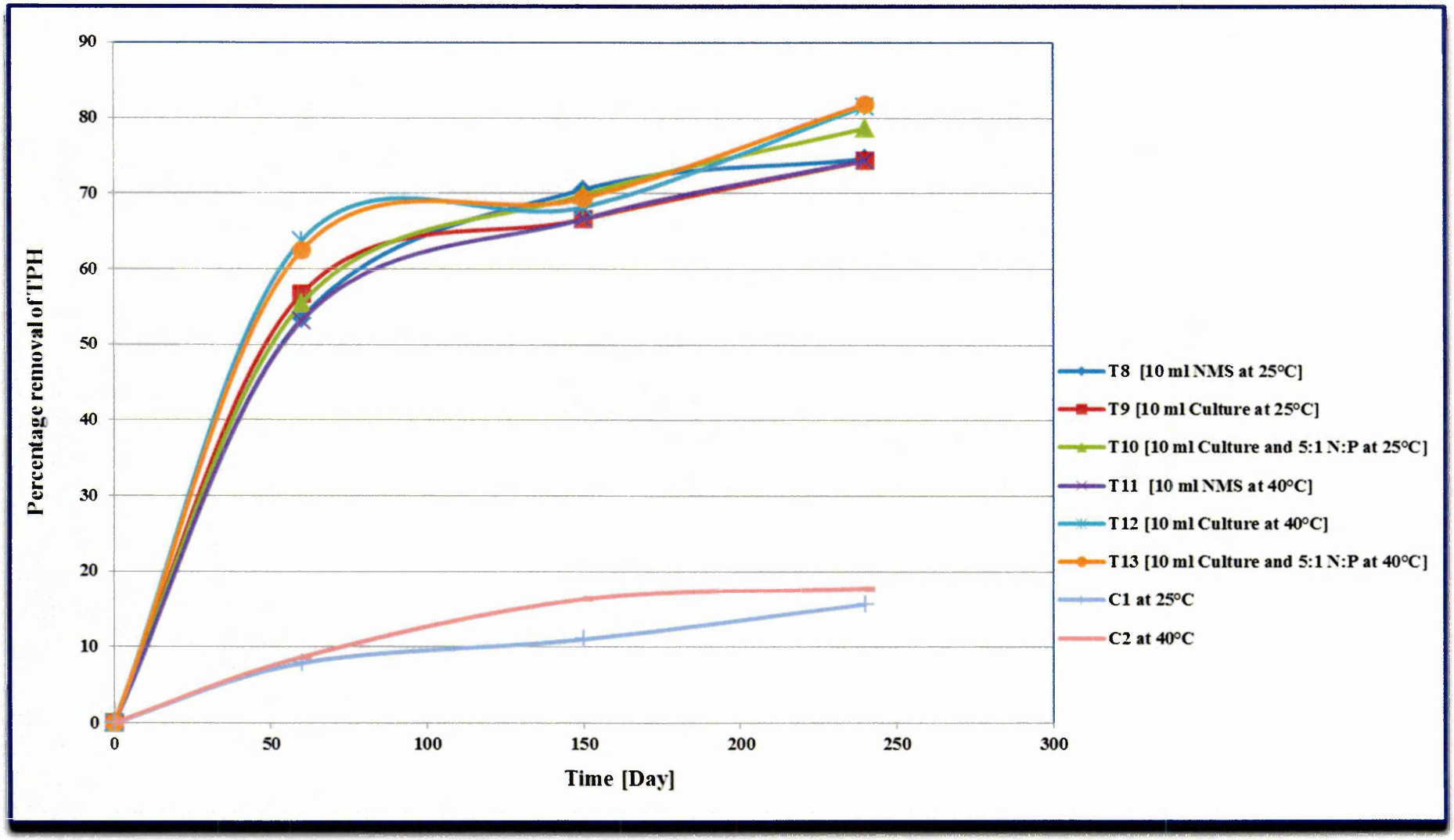


Figure 6-9 Percentage removal of TPH from soil during bioaugmentation constructed using the data from Figure 6-6

Generally the results show that degradation using bioaugmentation was greater in low crude oil contaminated soil samples compared to medium crude oil contaminated soil and high crude oil contaminated soil as follows: For low crude oil contaminated soil samples for treatment unit T34 (amended with culture plus 5:1 N:P at 25°C) in which 96.80 % of hydrocarbon was removed after 240 days of experiment period. The initial soil concentrations of hydrocarbon for treatments unit T34 was 8733.32 mg/kg soil at 40°C and reduced to 279.66 mg/kg soil. Medium crude oil contaminated soil for treatment unit T25 (amended with culture plus 5:1 N:P at 40°C) in which 95.11 % of hydrocarbons were removed. The initial soil concentration of hydrocarbon of hydrocarbon for treatment unit T25 was 37963.13 mg/kg soil was reduced to 1855.78 mg /kg soil. High crude oil contaminated soil for treatment T13 (amended with culture plus 5:1 N:P at 40°C) in which 81.84 % of hydrocarbon was removed over 240 days of treatments period. The initial soil concentrations of hydrocarbon for treatment unit T13 was 72266.89 mg/kg soil and was reduced to 13125.47 mg/kg soil. In addition, the lowest degradation rates in the bioaugmentation method were observed for low, medium and high crude oil contaminated soil samples for treatments unit T35 (amended with NMS only at 40°C) in which 91.86 % of hydrocarbon removed, T20 (amended with NMS only at 25°C) in which 91.17 % of hydrocarbons was removed and T11 (amended with NMS only at 40°C) in which 74.40 % of hydrocarbons removed respectively.

The analysis of extracted hydrocarbons from polluted soil by GC-FID is a powerful measurement to evaluate the biodegradation process. Oil biodegradation is also evident from the GC-FID scans of the alkane hydrocarbon fraction and significant reductions are observed in the alkane peak heights. Within the 240 days period, 73 % to 99 % of the total alkanes were removed, depending on the bioremediation method used.

Figures 6-10 A, B, C, D, E and F shows reperesentive examples of the chromatograms obtained from the GC-FID of some selected analysis treatment unit for day 0, 60, 150 and 240 plus overlay view of day 0 with 240 days for A) treated sample and (B) control samples. These chromatograms show that petroleum hydrocarbons were almost completely removed by biodegradation after 240 days of incubation. These results were validated by comparing the reduction of hydrocarbons in treated samples with control ones. The chromatograms for all other conditions are similar to the one shown in Figure 6-10A to Figure 6-10F. The chromatograms of samples at day zero represent the low and high molecular weights hydrocarbon and clearly illustrate the large number of compounds present in extracted hydrocarbon from the polluted soil at day zero.

Figure 6-10A shows the chromatograms of the hydrocarbon extracted from high crude oil contaminated soil from the Nasser Oilfield over time (0 , 60, 150 and 240 days) during microbial degradation experiments of sample which was incubated with the addition of water only,(sample code T2 at 25°C). Oil degradation is evident from the GC-FID scans of hydrocarbons and significant reductions were observed in the hydrocarbon peak heights as shown in the chromatograms over time. Within the 240 days period, 73.18 % of total petroleum hydrocarbon from the soil had been removed. The GC profile of the remnant hydrocarbon compounds (0 and 240 days overlay view) was compared with that of control under the same conditions (0 and 240 days overlay view) as show in Figure 6-10A (A and B) and these provided a clear indication of the degradation of hydrocarbon on soil.

Figure 6-10B shows the chromatograms of the hydrocarbons extracted from high contaminated soil from the Nasser Oilfield during microbial degradation in flask experiments. This sample (T4) was incubated with the addition of N:P 5:1 at 25°C. The data show that 79.92 % of hydrocarbon from the soil has been removed. From the chromatogram it can be seen that the highest degradation rates were obtained for longer saturated hydrocarbons and this is clear in comparison to the control one treated under the same condition. The reduction of higher molecular saturated hydrocarbons indicated that one of the strains of bacteria present in the soil consumed the long-chain hydrocarbons i.e. that they are biodegradable. Overlaying, and comparing chromatograms from treated soil over time (0 and 240 days) with that of control under the same conditions as show in Figure 6-10B (A and B) gives clear evidence of the biodegradation occurring.

Figure 6-10C shows the chromatograms of the hydrocarbons extracted from medium contaminated soil from the Nasser Oilfield over time during microbial degradation in flask experiments. This sample (T14) was incubated with the addition of water at 25°C. The data show that 93.38 % of hydrocarbon from the soil has been removed. From these data it can be seen that degradation was occurring in soil which is amended with the addition of water only. This indicates that under aerobic conditions, hydrocarbon degrading bacteria use part of the inorganic and organic nutrients present in soil. This agrees with Chaineau et al., 2005, and means that the bacteria found in polluted soil at the site tend to live in soil under site conditions and have the ability to break down the hydrocarbons from the soil using them as a source of carbon and energy. The chromatogram of the residual hydrocarbon remaining in the soil after 240 days of incubation was compared with the control treated under the same condition as the

samples as shown in Figure 6-10C (A and B). From these comparisons the hydrocarbon is clearly degraded in the soil.

Figure 6-10D shows the chromatograms of the hydrocarbons extracted from medium contaminated soil from the Nasser Oilfield during microbial degradation in flask experiments. This sample (T19) was incubated with the addition (5:1 N:P ratio) at 40°C. These data show that 98.50 % of the hydrocarbon from the soil has been removed. From the chromatograms it was observed that from day 150 to day 240, the biodegradation of hydrocarbons slowed down and seems to be no big difference to the unfertilized ones. The chromatogram of the residual hydrocarbon remaining in the soil was compared with the chromatogram of that remaining in the control; Figure 6-10D (A and B), it is clear that the biodegradation has occurred.

Figure 6-10E shows the chromatograms of the hydrocarbon extracted from lightly contaminated soil from the Nasser oilfield during microbial degradation in flask experiments. This sample (T26) was incubated with the addition (water only) at 25°C. These data show that 95.21 % of hydrocarbon from the soil has been removed. From the chromatogram it was observed that higher molecular weights of hydrocarbon are nearly completely degraded. In control sample the dose not show a longer reduction in total petroleum hydrocarbon after 240 days of incubation. The chromatograms presented in Figure 6-10E (A and B) shows the comparison between the GC profile of the treated soil and the GC profile of the control ones, and it is clear that the biodegradation has occurred.

Figure 6-10F shows the chromatograms of the hydrocarbon extracted from lightly contaminated soil from the Nasser Oilfield during microbial degradation in flask experiments. This sample (T28) was incubated with the addition (5:1 N:P ratio) at 25°C. These data show that 99.10 % of hydrocarbon from the soil has been removed. From the chromatogram it was observed that both low and high molecular weight hydrocarbons were nearly completely degraded. This was confirmed by comparing the chromatograms from treated soil and the control after 240 days of incubation as shown in Figure 6-10F (A and B).

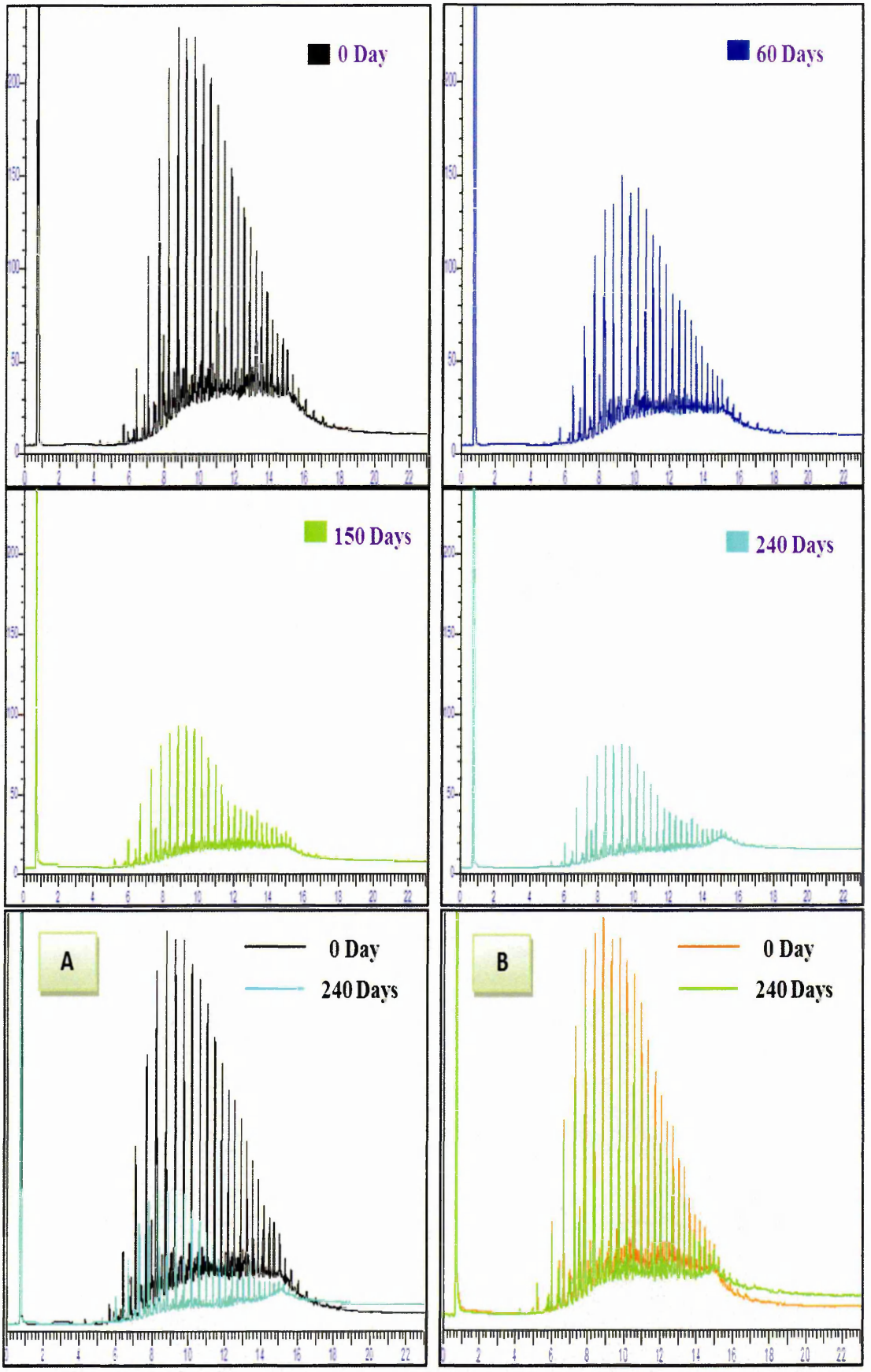


Figure 6-10A Chromatograms of extracted hydrocarbon from soil after biodegradation of soil contain high TPH concentration of sample code T2 (water added) plus chromatograms of (A) overlay view of zero and days 240 of treated polluted sample (B) overlay view of zero and days 240 of sterile control (C2)

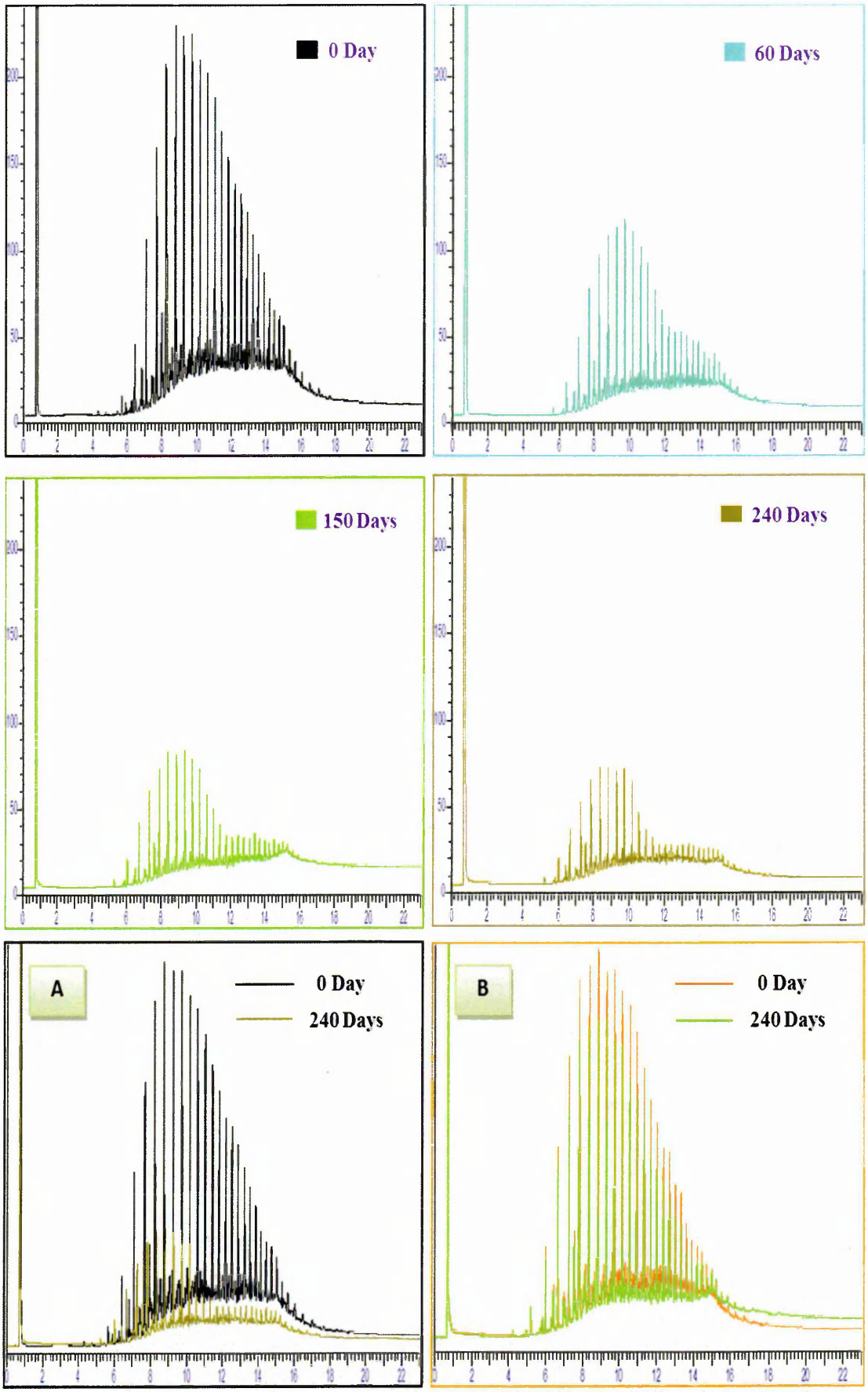


Figure 6-10B Chromatograms of extracted hydrocarbon from soil after biodegradation of soil contain high TPH concentration of sample code T4 (5:1 N:P added) plus chromatograms of (A) overlay view of zero and days 240 of treated polluted soil (B) overlay view of zero and days 240 of sterile control (C2)

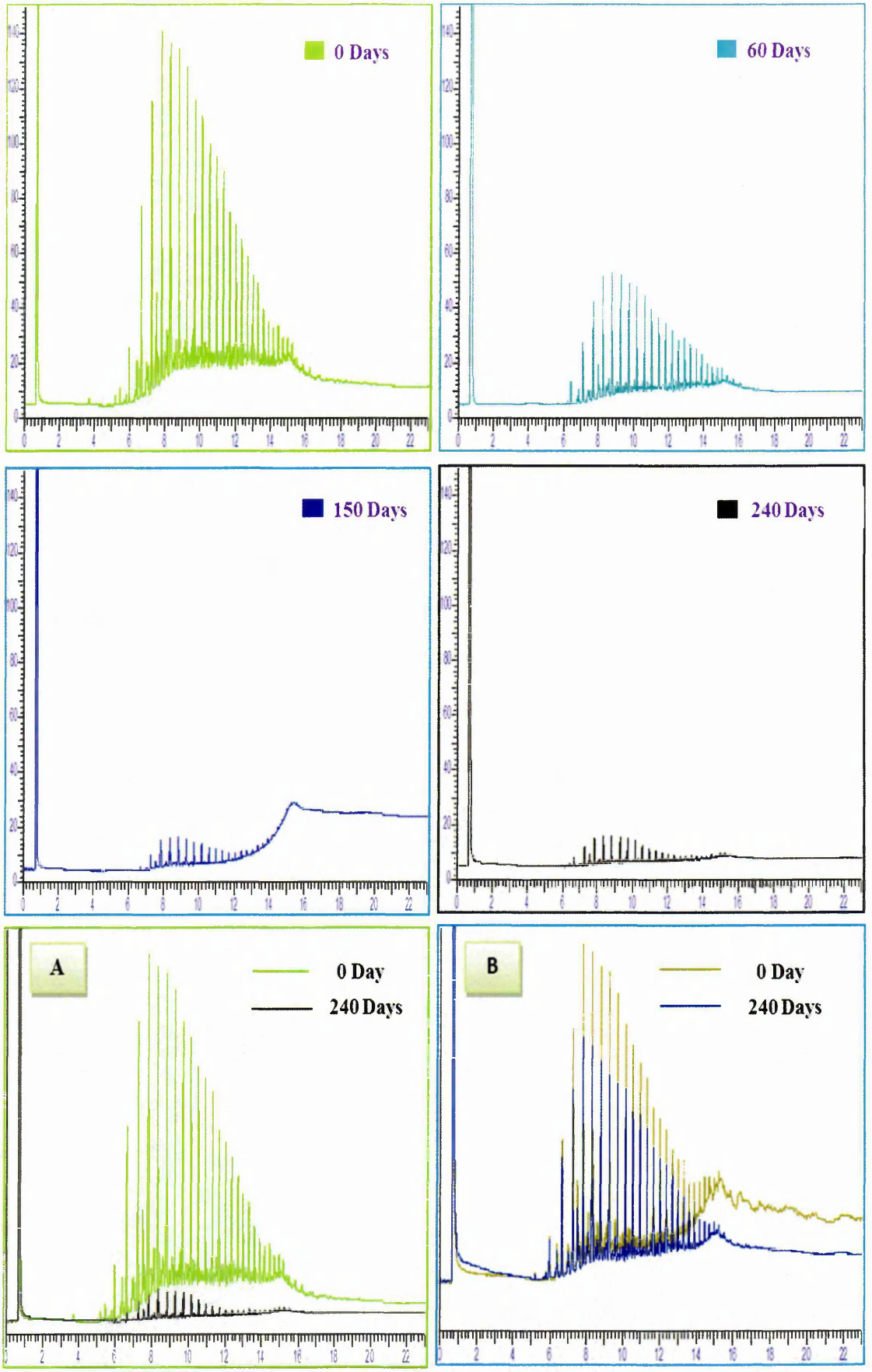


Figure 6-10C Chromatograms of extracted hydrocarbon from soil after biodegradation of soil contain medium TPH concentration of sample code T14 (water added) plus chromatograms of (A) overlay view of zero and days 240 of treated polluted soil (B) overlay view of zero and days 240 of sterile control (C4)

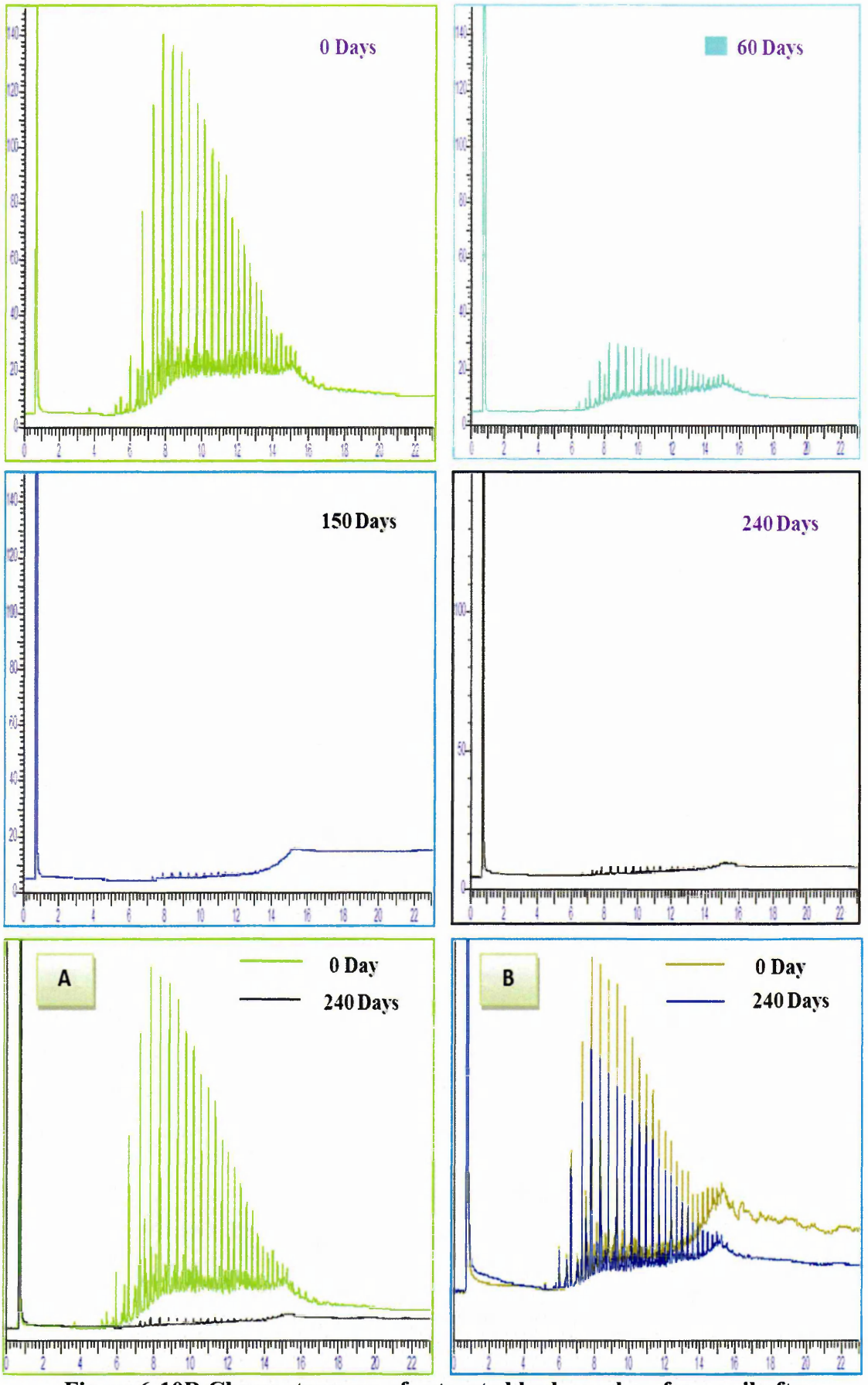


Figure 6-10D Chromatograms of extracted hydrocarbon from soil after biodegradation of soil contain medium TPH concentration of sample code T19 (5:1 N:P added) plus chromatograms of (A) overlay view of zero and days 240 of treated polluted soil B) overlay view of zero and days 240 of sterile control (C4)

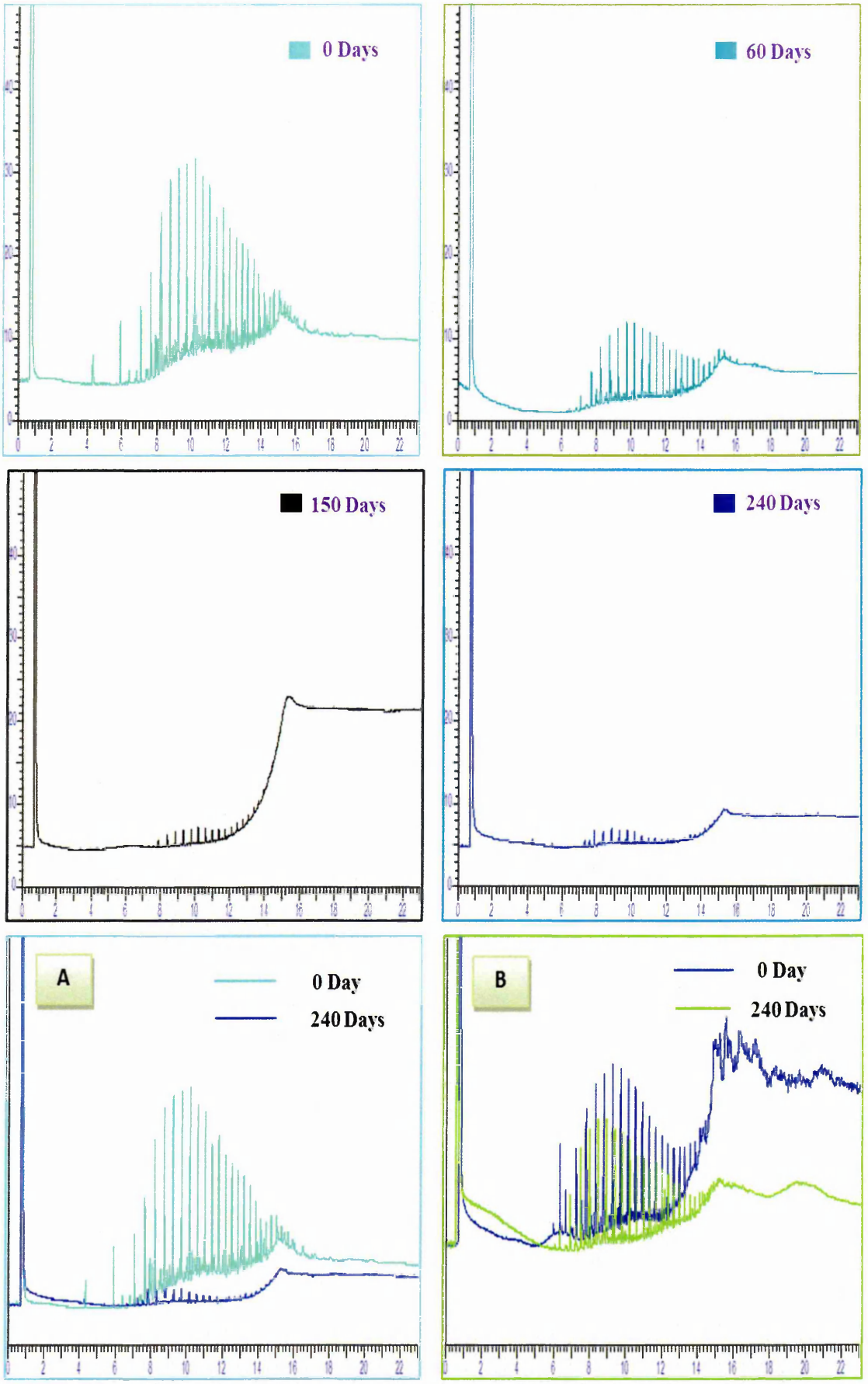


Figure 6-10E Chromatograms of extracted hydrocarbon from soil after biodegradation of soil contain low TPH concentration of sample code T26 (water added) plus chromatograms of (A) overlay view of zero and days 240 of treated polluted soil (B) overlay view of y zero and days 240 of sterile control (C6)

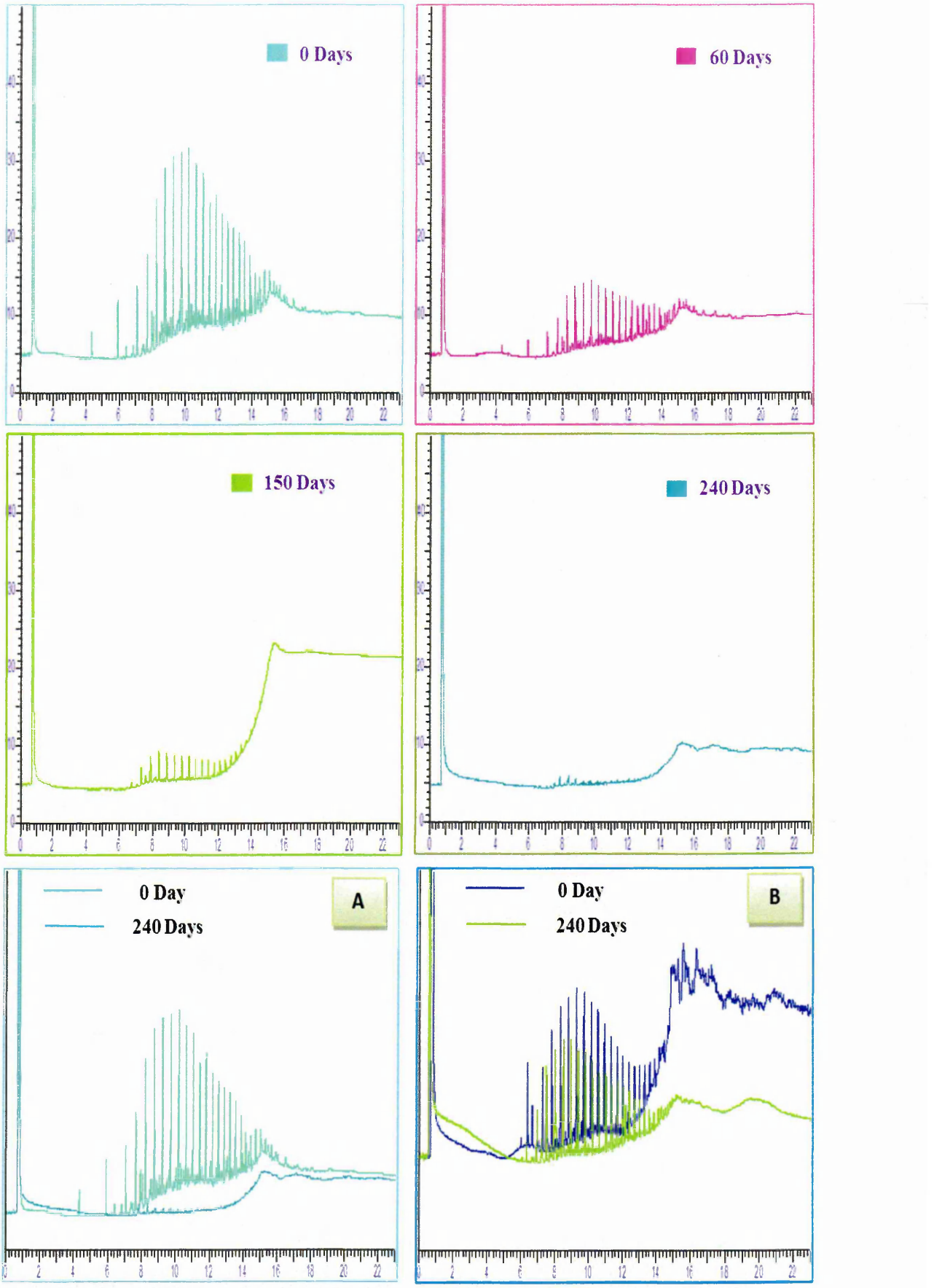


Figure 6-10F Chromatograms of extracted hydrocarbon from soil after biodegradation of soil contain low TPH concentration of sample code T28 (5:1 N:P added) plus chromatograms of (A) overlay view of zero and days 240 of treated polluted soil (B) overlay view of zero and days 240 of sterile control (C6)

6.12.2.3 Biodegradation of total petroleum hydrocarbon (TPH) from soil by adding 0.02% yeast extract

An additional set of biostimulation experiments was conducted in order to investigate the effect of adding a small concentration of organic nutrients in the form of yeast extract (6.7 mg per 100 g of soil). It should also be noted that the soil samples had been stored under ambient conditions for one year since the previous set of experiments, which may have caused changes in the microbial population and initial hydrocarbon content. The contaminated soil samples, A4 and A6, with TPH concentrations 3.37 % and 0.82 % respectively were used. All other experimental conditions were the same as those used previously. The laboratory biodegradation experiments were carried out in a 500 ml conical flask containing 250 grams of contaminated soil obtained from the disposal pit at the Nasser Oilfield, the soil moisture and pH were adjusted to 20-30 % of water holding capacity and 7.0 - 8.0 respectively. No further adjustment for other elements was made during the course of the study. The flasks were incubated at 40°C for 240 days in static incubator. For each soil TPH concentrations the following samples were used in duplicate two conditions were used in duplication as follows:

- Control- sterilised contaminated soil (150 g) +sterilised water (50 ml)
- Contaminated soil (150 g) + sterilised water (50 ml)
- Contaminated soil (150 g) + sterilised (0.02 % yeast extract solution (50 ml)

The flaskes were incubated in a static incubator under aerobic conditions for 240 days. During the experiment, soil samples were taken from the treatment flask and analysed periodically (0, 60, 150 and 240 days) for the determination of TPH, soil pH, soil moisture and microbial activities or availability. During the incubation time periodic renewal of the flasks was made by opening and aerating them (i.e. removing the cotton and aluminium foil from the flasks) for few minutes to allow sufficient oxygen to entire the flask.

Table 6-5 show the average total petroleum hydrocarbon concentrations (standard deviation of duplicate flasks are shown) over times in soil amended with water and 0.02 % yeast extract and the percentage of reduction in TPH concentration through 240 days of treatment. The results show that the highest hydrocarbon degradation rate was achieved in the first 60 days of incubation. The highest degradation rates occurred for treatment unit Y2 and Y4 (i.e. both amended with 0.02 % yeast extract) in which 79.51 % and 80.13 % respectively of hydrocarbon were removed from the treated polluted soil

samples. In the sterile control sample (C8) the maximum hydrocarbon removed was 11.59 % after 240 days of incubation as shown in Table 6-5.

Figure 6-11 and Table 6-5 shows the decrease in the average total petroleum hydrocarbon concentrations in low crude oil contaminated soil samples over the treatment time. These data show that the average concentration of total petroleum hydrocarbon removed from the polluted soil (Y4) amended with 0.02 % yeast extract was higher than the average concentration of total petroleum hydrocarbon removed from polluted soil (Y3) amended with water only. Both show significant differences to the sterile control. The initial average TPH concentration level of treated sample Y1 (i.e. amended with water) and Y2 (i.e. amended with 0.02 % yeast extract) were 8211.81±38.46 mg/kg soil and 8461.39±199.24 mg/kg soil which reduced to 3207.70±522.72 mg/kg soil and 1735.29±131.93 mg/kg soil respectively after 240 days of treatment. The highest biodegradation rate was recorded for soil sample Y2 (i.e. amended with 0.02 % yeast extract) in which 79.51 % of hydrocarbon from the polluted soil was removed after 240 days of treatment. While in sterilized control sample (C7) treated under the same condition as the samples the hydrocarbon concentrations was reduced by 8.69 % after 240 days of treatment. Again the degradation of TPH in the soil amended with water only was less than the soil amended with 0.02 % yeast extracted.

Figure 6-12 shows the chromatograms of the hydrocarbon extracted from low crude oil contaminated soil of the Nasser Oilfield over time (0 , 60, 150 and 240 days) after microbial degradation in flask experiments of sample which amended with 0.02 % yeast extract at 40°C. The result shows that 79.51 % of hydrocarbon from the soil has been removed. From the chromatogram it was observed that the hydrocarbon was degraded over time of treatment. The degradation of hydrocarbon was confirmed by comparing the chromatogram of hydrocarbon extracted from the treated polluted soil and the chromatogram of the extracted hydrocarbon from the control samples as shown in Figure 6-12 (A and B). The sterile control shows that small reduction in total petroleum hydrocarbon after 240 days of incubation comparing with zero days and the maximum percentage of hydrocarbon reduction was 8.69 % after 240 days of incubation.

Figure 6-13 shows the reduced of total petroleum hydrocarbon concentrations in medium crude oil contaminated soil samples over the treatment period. The data show that the highest degradation rates were observed in the first 60 days of treatment in two of the treatment conditions. In polluted soil (Y3) amended with water only the total extent of hydrocarbon biodegradation was 58.26 % after 240 days of treatment whereas 80.13 % of the hydrocarbon was degraded in polluted soil (Y4) amended with 0.02 %

yeast extract over the same period. The initial average concentrations of total petroleum hydrocarbon in both soil samples Y3 (i.e. amended with water only) and Y4 (i.e. amended with 0.02 % yeast extract) were 33377.18±1457.52 mg/kg soil and 33643.72±112.74 mg/kg soil respectively. These were reduced to 13923.07±147.60 mg/kg soil and 9683.31±6358.78 soil mg/kg respectively. The highest biodegradation rate was observed in sample Y4 in which 80.13 % of hydrocarbon from the polluted soil was removed after 240 days of treatments, whereas, only 11.59 % of the initial hydrocarbons were eliminated in sterile control samples (C8) as shown in Figure 6-13 and Table6-5.

Figure 6-14 shows the chromatograms of the hydrocarbons extracted from medium crude oil contaminated soil of the Nasser Oilfield over time (0 , 60, 150 and 240 days) after microbial degradation in flask experiments for samples amended with 0.02 % yeast extract at 40°C. These data along with visual observation showed that lower to higher hydrocarbons were nearly completely removed. The data show that 80.13 % of hydrocarbon from the soil has been removed. The sterile control shows small reduction in total petroleum hydrocarbons after 240 days. The total hydrocarbon reduced in sterile control (C8) was 11.59 %. The chromatogram presented in Figure 6-14 (A and B) shows the comparison between the GC profile of the treated polluted soil and the GC profile of the sterile control one, and it is clear from both chromatograms that the biodegradation of hydrocarbons has occurred.

Table 6-5 Average total petroleum hydrocarbon (TPH in mg/kg) removed from the soil in treatment unit (Mean and standard deviation of duplicate samples are shown)

Time / Microcosm ID	0 Day	60 Days	150 Days	240 Days	RED. (%)
C7	7818.69 ±159.01	7455.36 ±86.85	7177.47 ±50.64	7137.38 ±25.17	8.69
Y1	8211.81 ±38.46	5001.44 ±242.77	4099.59 ±898.29	3207.78 ±522.72	60.95
Y2	8461.39 ±199.24	4373.81 ±53.07	3271.04 ±248.05	1735.29 ±131.93	79.51
C8	31146.37 ±331.55	29908.83 ±305.10	27800.71 ±187.60	27537.08 ±88.32	11.59
Y3	33377.18 ±1457.52	20313.61 ±478.66	15560.74 ±1492.61	13923.07 ±147.54	58.25
Y4	33643.72 ±112.74	15592.07 ±1249.27	13753.13 ±769.54	6683.31 ±2116.51	80.13

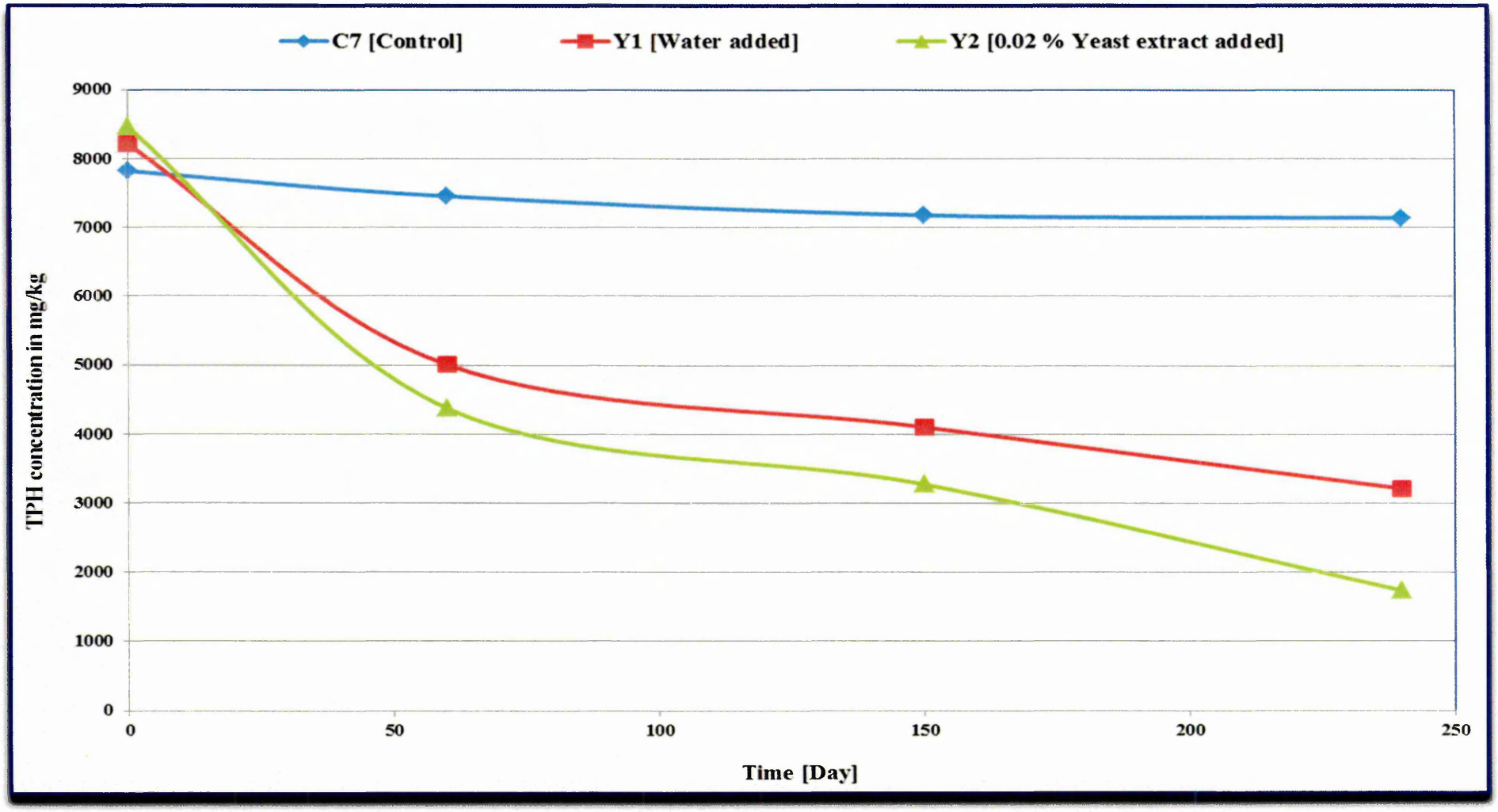


Figure 6-11 Reduction in TPH concentrations in treated polluted soil (low TPH in mg/kg) and sterile control sample (C7) over time

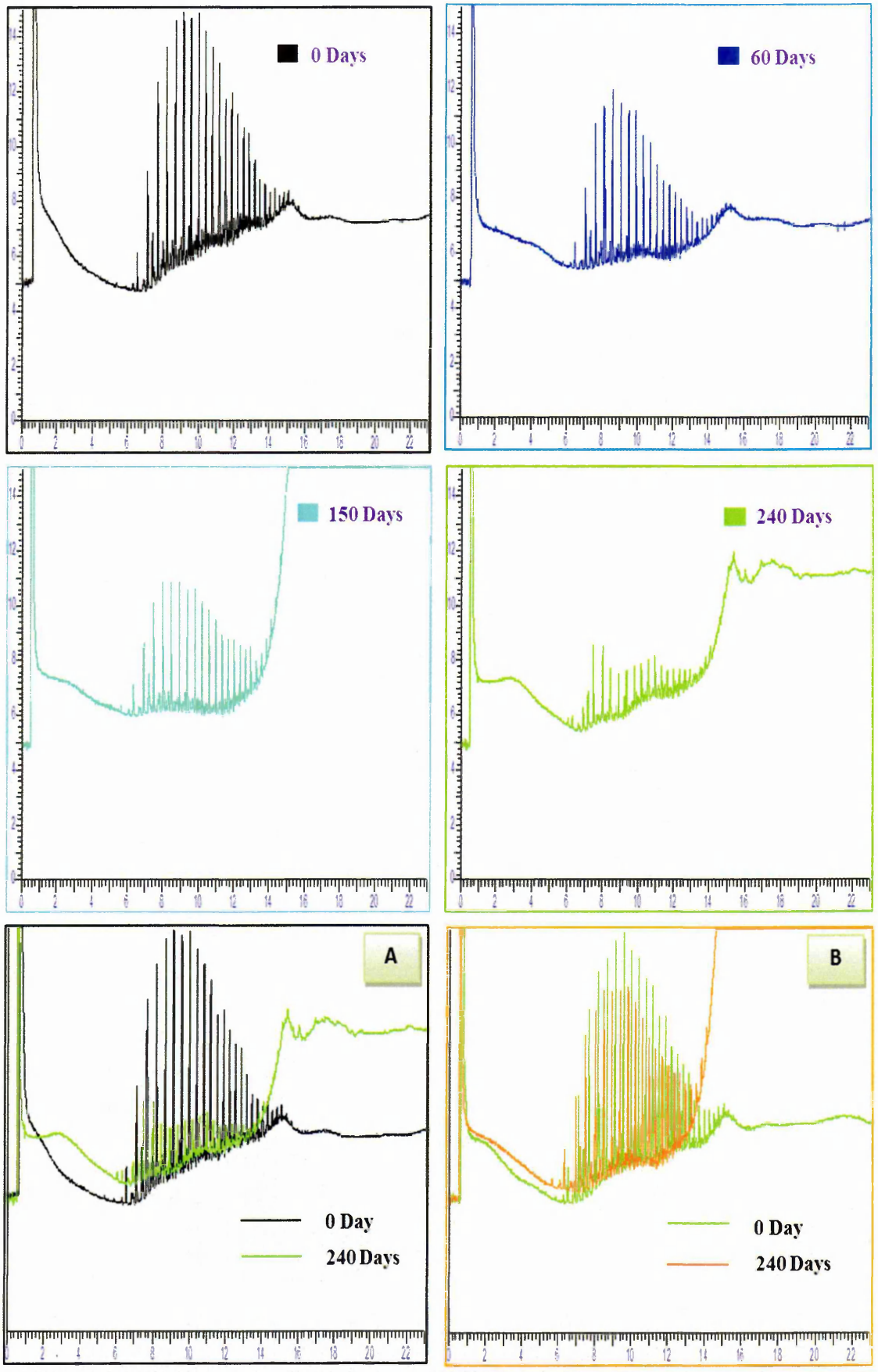


Figure 6-12 Chromatograms of extracted hydrocarbons from contaminated soil over time show the biodegradation of crude oil in the soil of low TPH concentration of sample A6 amended with 0.02 % yeast extract and the chromatograms of (A) overlay view of zero and days 240 of treated polluted soil (B) overlay view of zero and days 240 of sterile control (C7)

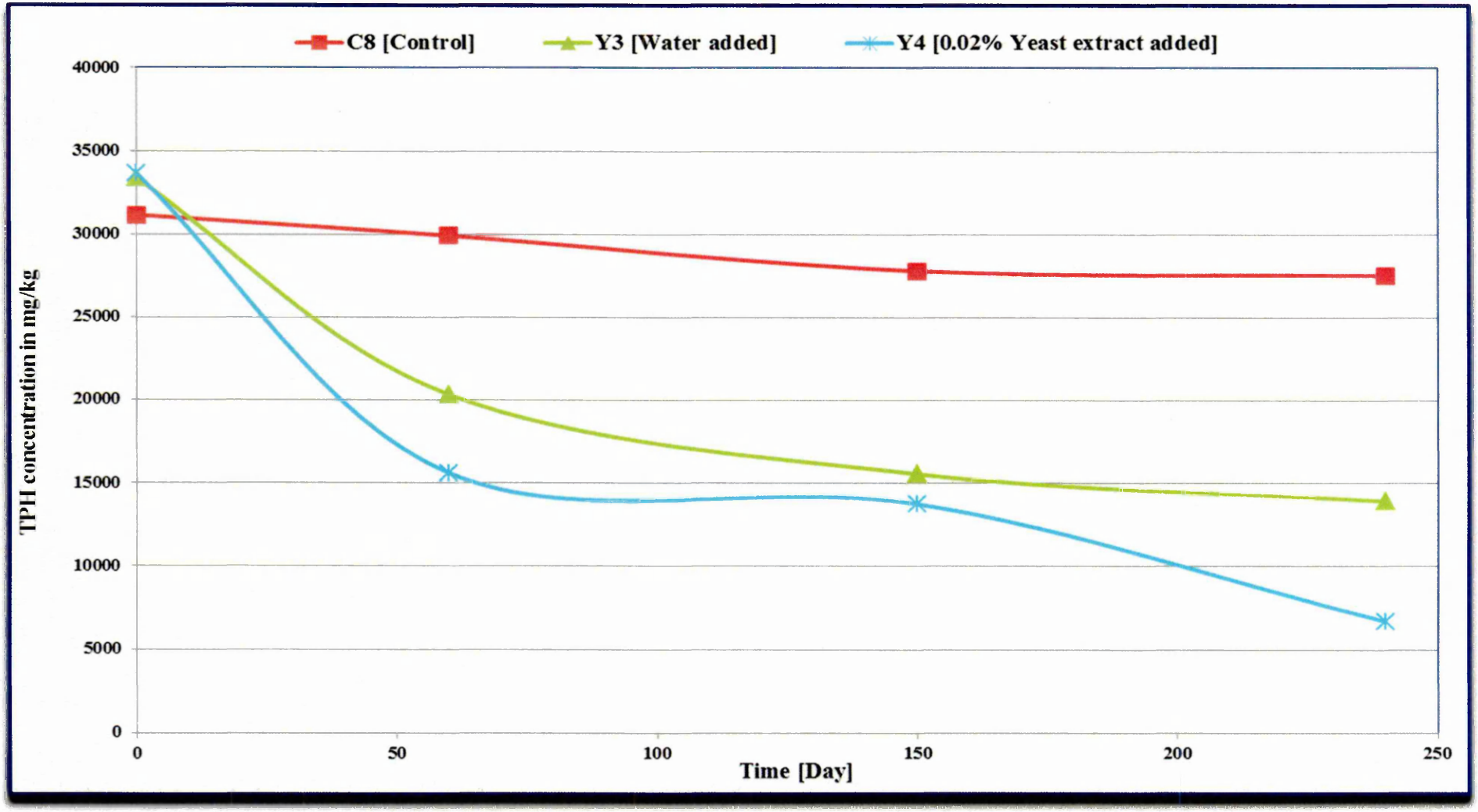


Figure 6-13 Reduction in TPH concentrations in treated polluted soil (medium TPH in mg/kg) and sterile control (C8) over time

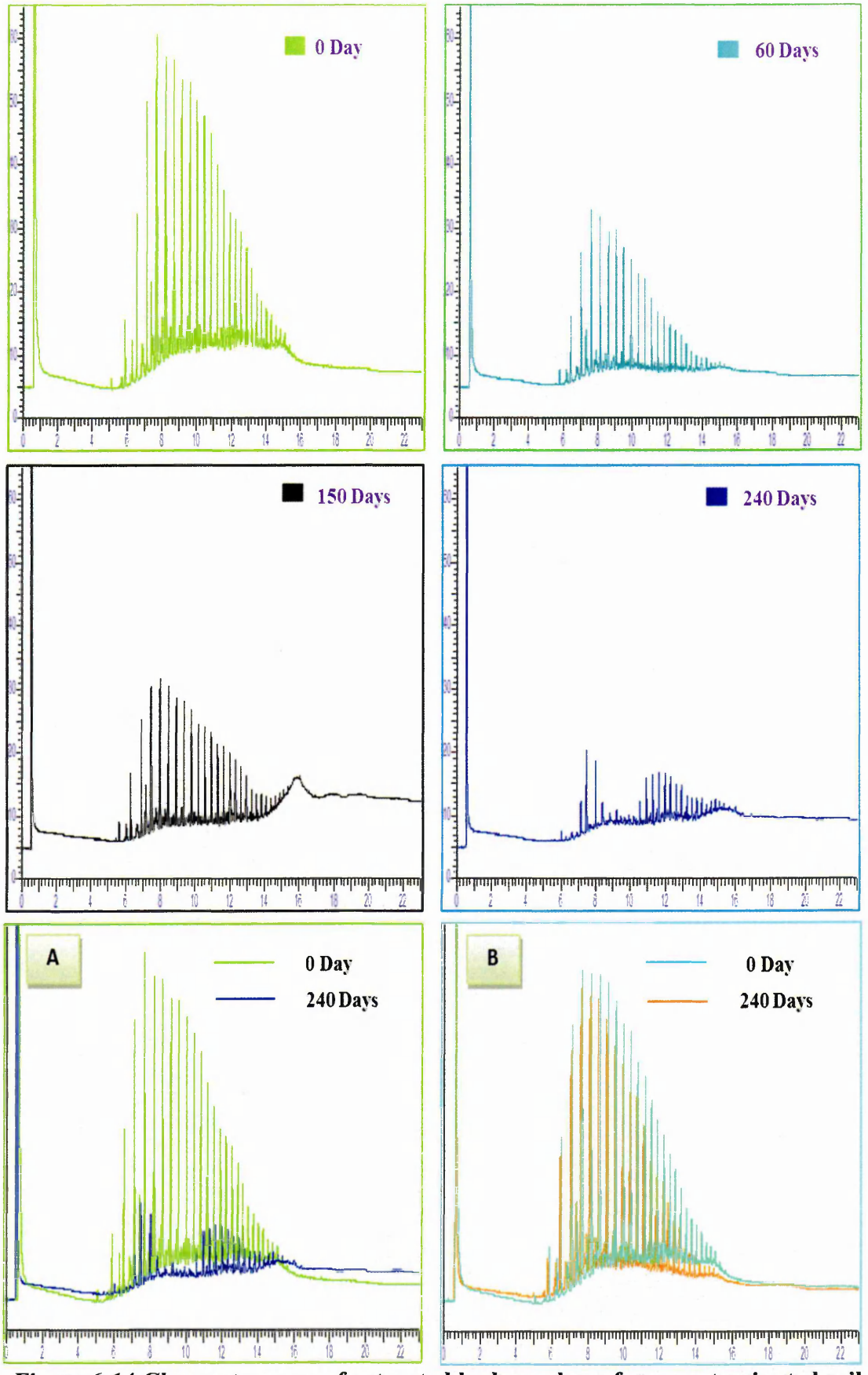


Figure 6-14 Chromatograms of extracted hydrocarbons from contaminated soil over time show the biodegradation of crude oil in the soil of medium TPH concentration of sample A4 amended with 0.02% yeast extract and the chromatograms of (A) overlay view of zero and days 240 of treated polluted soil (B) overlay view of day zero and day 240 of sterile control (8).

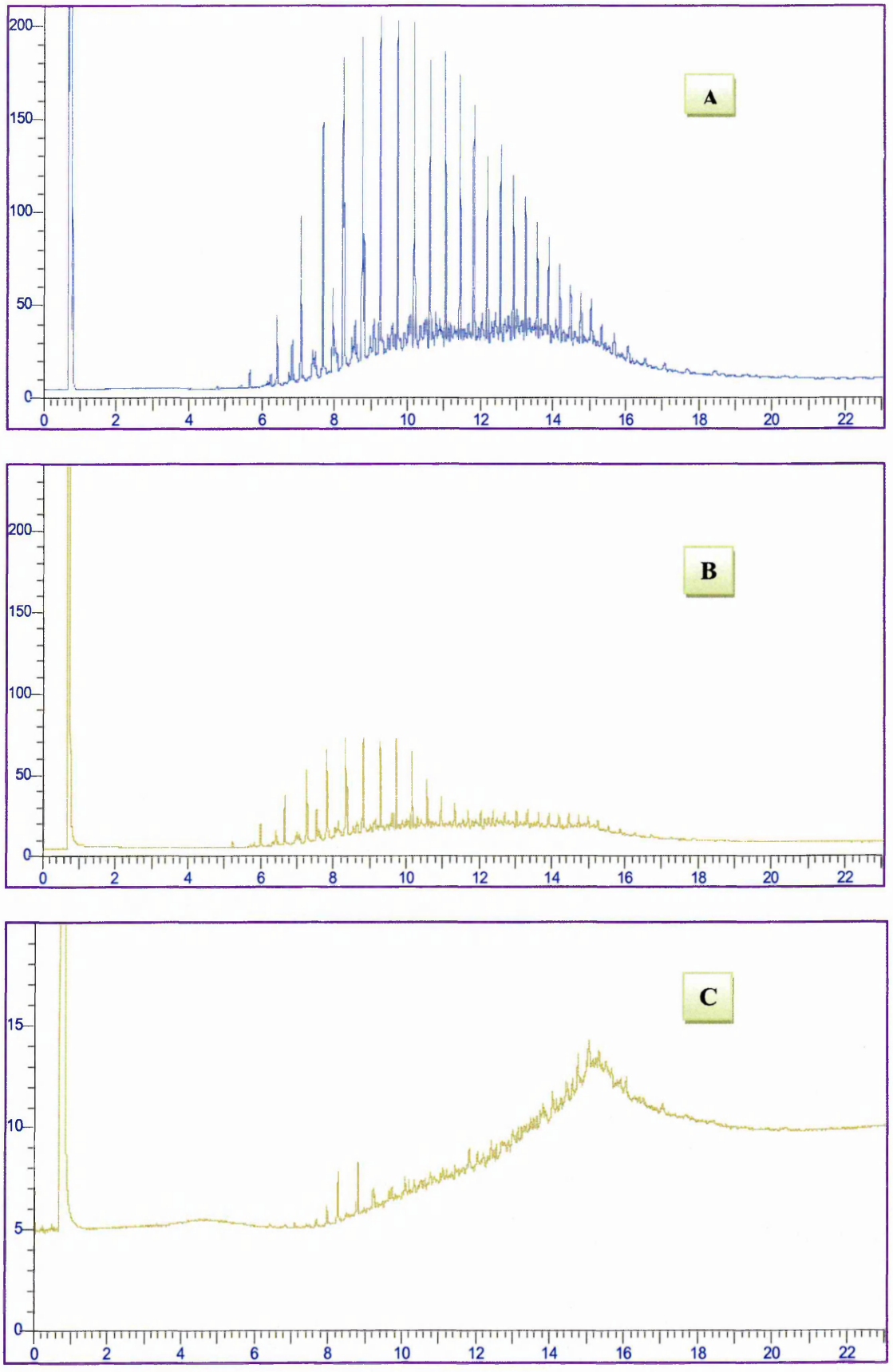


Figure 6-15 Representative chromatogram of A) the fresh crude oil extracted from polluted soil, B) the crude oil extracted from polluted soil after 240 days of incubation and C) aged contaminated polluted soil (20 years old)

The chromatograms obtained from all samples at various times of treatment showed that some of the hydrocarbons were degraded to different extents / had different depletion rates. For comparison purposes, the chromatograms of the hydrocarbons extracted from fresh crude oil polluted soil (zero day) and that from treated soil after 240 days of incubation (enhanced method) and that from the dry weathered soil sample aged 20 years taken from the dry oily produced water disposal pit at the Nasser Oilfield were compared. This enabled comparison of the impact of the hydrocarbon type and concentration, as well as the total effect of the natural weathering process on hydrocarbon biodegradability in soil.

Figure 6-15 shows representative chromatograms of the fresh crude oil extracted from soil (which caused the contamination of the soil), crude oil extracted from soil after 240 days of incubation (enhanced method) and crude oil extracted from aged crude oil contaminated soil after 20 years of contamination. This remaining fraction of crude oil in the aged polluted soil can represent the natural environmental endpoint (Maletic et al., 2011). During the weathering process, carbon derived from contaminants becomes incorporated into the soil as natural organic matter, due to the chemical oxidation reactions as well as biological activity. Slow diffusion into very small pores in particles within the soil, sorption onto the organic matter and the formation of semi-rigid films around non-aqueous-phase liquids, all cause a high resistance to mass transfer out of the soil (i.e. evaporated to the air and/or penetrate down through the soil). However, the weathered fraction can represent an environmentally acceptable endpoint. The results from the current study show that the reduction of total petroleum hydrocarbons in fresh crude oil polluted soil samples reaches up to 85.32 % after 240 days, where in the aged polluted soil samples 96.46% of hydrocarbon was removed in 20 years by natural attenuation. If we assume that the hydrocarbon reduction in the aged polluted soil sample has occurred via natural microbial activity and not simply weathering condition, in this study the amounts of hydrocarbons lost by enhanced biodegradation are significant within 240 days. The data show that the degree of attenuation of hydrocarbon contamination achieved 240 days in this study was comparable to that achieved in 20 years in the original environment.

Trindade et al., 2005, carried out a bioremediation study comparing weathered (4 years old) and recently oil contaminated soil samples from Brazil. They concluded that bacteria from the weathered soil performed better than the ones in the freshly contaminated soil. They also found that bioaugmentation and biostimulation doubled the bioremediation efficiency achieved by natural attenuation. The current study shows

that the reduction of hydrocarbon from contaminated soil achieved in 240 days using biostimulation and bioaugmentation were comparable to that achieved from weather polluted soil in 20 years in the original environment.

6.13 Discussion

The continuous disposal of oily PW onto the surface of the open environment causes great damage to the ecosystem. Thus, it is necessary to develop technologies designed to remediate hydrocarbon contamination in soil using naturally compatible and inexpensive technologies. These have to be designed and tested thoroughly before application. In this way, the use of microbes already existing in the soil provides a useful alternative for the bioremediation of soils contaminated with petroleum hydrocarbon. Bioremediation is the process of using living organisms or their constituents to breakdown the molecular structure of organic contaminants into substances or compounds that are not hazardous to humans and to the environment. In this study, biostimulation and bioaugmentation at a lab scale have been used as bioremediation techniques for a crude oil contaminated soil from the Nasser Oilfield, Libya. The concentrations of TPH in highly oil contaminated soil samples were reduced from 72266.89 mg/kg to 10607.05 mg/kg and from 72266.89 mg/kg to 13125.47 mg/kg after 240 days of incubation under biostimulation and bioaugmentation respectively. The concentrations of TPH in moderately oil contaminated soil sample were reduced from 37963.13 mg/kg to 713.61 mg/kg and from 37963.13 mg/kg to 1855.78 mg/kg after 240 days of incubation under biostimulation and bioaugmentation respectively. The concentrations of TPH in moderately oil contaminated soil sample amended with 0.02% yeast extract were reduced from 33643.72±112.74 mg/kg to 6683.31±2116.51 mg/kg after 240 days of incubation under biostimulation respectively. The concentrations of TPH in lightly oil contaminated soil sample were reduced from 8733.32 mg/kg to 78.29 mg/kg and from 8733.32 mg/kg to 279.66 mg/kg after 240 days of incubation under biostimulation and bioaugmentation respectively. The concentrations of TPH in lightly oil contaminated soil sample amended with 0.02% yeast extract were reduced from 8461.39 mg/kg to 199.24 mg/kg after 240 days of incubation under biostimulation method. In this study the biodegradation using biostimulation ranged from 73.17% to 99.10 % after 240 days of incubation. The biodegradation rates using bioaugmentation techniques ranged from 74.40 % to 96.80% under the same time course of experiments. The highest TPH degradation rate was observed in the first 60 days of incubation in both treatments methods used and slowly continued up to the 240 days of the experiment. In general

the results obtained from the biostimulation and bioaugmentation methods indicated that the contaminated soil from the Nasser Oilfield is biotreatable, and hydrocarbons in soil may be reduced with up to 99 % efficiency after 240 days of incubation. Biological technologies have been successfully applied for remediation of soils highly contaminated with hydrocarbons. According, to Atlas 1981, Leahy et al., 1990, Mancera-Lopez et al., 2008 and Maletic et al., 2011, the level of hydrocarbons biodegradability will depend on the composition of hydrocarbons and microflora available in the environment. Many other environmental factors such as oxygen, temperature, nutrients, pH, moisture content, etc., may influence the bioremediation processes and must be taken into account (Abu et al., 2008, Mercer et al., 2011, and Das et al., 2011).

In the present study an environment suitable for microbial activity was provided by stimulating the temperature of the contaminated site (i.e. 25°C to 40°C), maintaining moisture content at 20-30% of water holding capacity and turning the soil for proper aeration. In addition, the pH was adjusted between 6 to 8 which is favourable for bioremediation. According to Liu et al., 2009, and Mercer et al., 2011, temperature is an important factor for controlling microbiological activity and the rate of hydrocarbons degradation in soil. Degradation can occur at wide range of temperatures with the most rapid rate occurring between 30 and 40°C. Horel et al., 2009 has studied the effect of temperature and moisture content on biostimulation of diesel oil using fertilizer as a stimulating agent. They concluded that the bioremediation of hydrocarbons started much earlier at a higher temperature than at lower ones.

On the other hand, the moisture content and regular mixing to enhance soil porosity did not influence degradation significantly. Liu et al., 2001 found that the biodegradation of phenanthrene increased about 50 % as the soil water content increased. In the present study the above parameters (i.e. pH, temperature and moisture content) were adjusted and monitored during the experiment period. The results show that there are no effects on the microbial activity for degrading hydrocarbons from the soil at the site. This means that the microorganisms present in soil adapted to or tolerated to the environmental conditions especially high temperature and were capable of removing hydrocarbons from soil. From this study it is clear that by providing suitable environment conditions effective bioremediation of crude oil contaminated soil on the Nasser Oilfield could be achieved and may also reduce the time required to remediate contaminated soil. There is however other parameters that have a considerable effect on the biodegradation of hydrocarbons such

as soil salinity and nutrients present. Bioremediation of polluted soil may be enhanced by adding nutrients such as nitrogen and phosphorus with suitable microbial populations. The result shows that the high salt content in soil does not affect the hydrocarbons removed. This means that the microorganisms present in soil adapted to and tolerated the environmental conditions and were capable of removing hydrocarbons from soil under high soil salinity. According to Margesin et al., 2001 soil bioremediation experiments involve the use of sterilized fresh water over an extended period of time to maintain the moisture content; this leads to a reduction in the soil salinity. The effectiveness of bioremediation of saline soils that are polluted by hydrocarbons is dependent on the ability of natural microflora to tolerate the levels of salinity in the environment. Furthermore, the type and number of substrates that can be utilized by microorganisms may be decreased as the salinity increases. At high salinity hydrocarbon degradation is often restricted to n-alkanes (Obuekwe et al., 2005 and Betancur-Galvis et al., 2006). According, to Leahy et al., 1990 the ability of microorganism to breakdown the hydrocarbons decreases under high salinity. In this study, the isolated strain from the soil at the study site was shown to have the ability to breakdown the hydrocarbon under high salt contents. This is mean that the microbes present in soil adapted and tolerate to the environmental conditions. However, the ability of microorganisms to degrade hydrocarbon under salt content soil environments are expected to be salt adapted (Betancur -Galvis et al., 2006).

Jackson et al., 1999, investigated the biodegradation of crude oil on Louisiana salt marsh soil. They found low degradation rates (0 - 3.9 % per day) for alkane (i.e. C_{11} and C_{17}) and high degradation rates (8 - 16 % per day) for PAHs (naphthalene, C1, and C2-Naphthalene and Phenanthrene, C1, and C2-Phenanthrene). They also found by nutrient addition to the crude oil contaminated soil successful enhanced degradation of many alkanes and total PAHs. This rate is comparable to the rates of hydrocarbon removal observed during the current study.

Rhykerd et al., 1995 applied bioremediation to soil contaminated with oil and salt as a result of spill from oil production near Refugio and college station, Texas, USA. They studied the effect of salt (NaCl) on degradation of oil in soil, they treatment soil solution adjusted to 40, 120 and 200 dS/m electrical conductivity and amended with nutrient (i.e. soils amended with nitrogen at 0.75 g of NH_4NO_3 per kg soil and phosphorus at 0.16 g of K_2HPO_4 per kg soil). They reported that 25–44% of the initial concentration (50,000 mg/ kg) of lubricant oil degraded during 128 days at 25°C. They found that a high salt concentration (200 dS/m) in soil may result in a decrease in the

oil removed by 25 % comparing to the low salt concentration (40 dS/m) reduced oil by 35 % from the initial concentration. They conclude that the bioremedation of oil in soil was reduced by salinity. They conclude that the removing the salts from contaminated soil before undertaking bioremediation reduces the time of treatment.

Obuekwe et al., 2005 investigated the hydrocarbon utilization and degradation under high temperature and saline conditions of crude oil contaminated soil using three fungi isolated from a salt marsh in the Kuwaiti desert. They found that the three isolated fungi from a Kuwaiti saline environment can extensively degrade the *n*-alkane fractions of crude oil under saline and high temperature conditions. They concluded that even under high salts/temperature conditions provision of nutrients will be beneficial for bioremediation of petroleum polluted environments.

Betancur -Galvis, et al., 2006, investigated the effect of mineral nutrients, N and P, which contains large amounts of nutrients and organic material, in the restoration of hydrocarbon-contaminated alkaline–saline soil taken from former Lake Texcoco in the valley of Mexico City, Mexico. They found addition of N:P to both soils little increased the initial biodegradation rate of PAHs after 112 days. They conclude that lower degradation rate in alkaline saline soil of Texcoco compared to normal soil. However, in the present study little difference was observed in soil amended with nutrients compared with soil amended with water only. This indicates that, in the soil from the site studied here, availability of nutrients in the soil is not a substantial rate-limiting factor on the process of oil biodegradation.The current study also shows that, despite the high soil salinity of the site studied, an acceptable biodegradation rate can be achieved. It is expected that the indigenous microorganisms in this environment are salt-adapted.

As detailed in the introduction the addition of nutrient has been reported to improve the efficiency of bioremediation by increasing the rate of degradation (Atlas 1995). However, in the present study little difference was observed in soil amended with nutrients compared with soil amended with water only, from which it can be concluded that the microbes present within soil may be using the nutrients already available in the soil and that the availability of such nutrients is not a major limiting factor on the progress of hydrocarbon oxidation.

Sarkar et al., 2005 studied the addition of N and P to enhance the biodegradation of diesel contaminated soils and compared this to natural attenuation. The authors concluded that up to 96 % of hydrocarbons were removed from diesel contaminated

soil (3350 mg/kg soil) after the addition of inorganic fertilizers (N and P) to diesel contaminated soils, while 93.8 % of hydrocarbons were removed in unfertilized ones. The results from this study are similar to the present investigation in that a small increase in biodegradation was observed as a result of adding additional nutrients, but biodegradation also proceeded well in the absence of fertiliser.

Lee et al., 2007 has examined the effect the addition of nutrient to soil contaminated with waste lubricants under different nutrient conditions over a 105 day testing period. They concluded that bioremediation using biostimulation is a viable choice for the remediation of soil contaminated with waste lubricants. They found that when nutrients are added to the polluted soil a substantial stimulation of hydrocarbon bioremedation occurred in fertilized for hydrocarbon degradation compared to non-fertilized soil. Chorom et al., 2010 investigated whether agricultural fertilizers (N, P) enhance the microbial degradation of petroleum hydrocarbons in soil by using artificially polluted soil with 1 % of crude oil. They found that the addition of fertilizer increased the degradation of the hydrocarbons. Furthermore, they found a reduction in hydrocarbons in the range of 45 to 60 % in all treatments after 5 weeks of experiment.

Ruberto et al., 2003 carried out biostimulation and bioaugmentation of soils polluted with 14,380 mg/ kg TPH under natural conditions. The authors found that 75 % of TPH removal using bioaugmentation when compared to the biostimulation treatment in which 65 % of hydrocarbons were removed from polluted soil during 56 days of experiments. However, they showed that using biostimulation and bioaugmentation with the isolated strain from soil improved the contaminant elimination when other parameters are adjusted. This is another example of system where a relatively modest improvement in biodegradation is possible via biostimulation or bioaugmentation.

Sarkar et al., 2005 studied the bioremediation of diesel contaminated soils by comparing a method of natural attenuation with two methods of biostimulation. The results revealed that biodegradation of petroleum hydrocarbons was enhanced using two methods of biostimulation by up to 96 % when compared to natural attenuation.

Bento et al., 2005 compared three bioremediation technologies (natural attenuation, biostimulation and bioaugmentation) to treat soil polluted with diesel oil. They concluded that the best and highest degradation rate of diesel oil occurred using bioaugmentation comparing with other two methods. Milic et al., 2009 examined the composition of the microbial consortium during the bioremediation of heavy contamination of soil by crude oil taken from Pancevo Oil Refinery, Serbia using biostimulation and bioventilation methods. They found that 89 % of TPH was

removed after 5.5 months of biodegradation experiments. As mentioned previously in the present study TPH degradation rate under controlled ratio of N: P (i.e. 5:1 and 10:1) nutrition was observed with little difference with unfertilized soil. In addition, Khasbayar et al., 2010 carried out bioremediation of soils polluted with crude oil. They found that 80 % of TPH in soil contaminated with complex mixture of hydrocarbons amended with culture was removed. Furthermore, Liu et al., 2010 carried out a study on bioremediation of oily sludge contaminated soil by biostimulation of indigenous microbes for 360 days at the Shengli Oilfield in northern China with 241000 mg/kg of TPH concentration. They found that 58.2 % of the total petroleum hydrocarbon (TPH) was removed from the treated soil compared with only 15.6 % in the control sample.

Maletic et al., 2011 investigated the impact of the hydrocarbon type, concentration and the total effect of natural weathering on the biodegradability of hydrocarbons in soil. They carried out biodegradation experiments in soil contaminated with diesel and crude oil collected from the oil refinery dumping site after 9 years of weathering. The biodegradation process was monitored by measuring, CO_2 evolution rate, hydrocarbon degradation rate and dehydrogenase activity. The authors found that in the case of diesel contaminated soil the best concentration was 1 %, with concentrations of about 2 % causing a slightly adverse effect on the CO_2 production after 2 weeks, while a 3.5 % concentration of diesel caused significant toxicity. For soil contaminated with crude oil, 2.0 % was found to be optimum for effective biodegradation, with 3.5 % crude oil also causing adverse effects to CO_2 production. For weathered soil no adverse effect was obtained for any concentration after the weathering process, the remaining contaminants in the soil were mostly poorly degradable constituents. They concluded that hydrocarbon biodegradability strongly depends upon the concentration, type and weathering of the hydrocarbons.

The addition of adapted bacteria to soil (bioaugmentation) has shown a limited increase in the overall efficiency of hydrocarbon removal when compared with the control without added bactteria. The addition of organisms can accelerate the initial phase of biodegradation and can be advantageous when contaminants have a toxic effect on the indigenous microorganisms.

Liu et al., 2011 has studied the effect of bioremediation by using bioaugmentation (selected consortium and kitchen waste) and biostimulation [N:P 27:6.5 (i.e 3 g N + 0.45 g of P per kg soil from NH_4NO_3 and K_2HPO_4) and N:P 11:3.7 (i.e 0.85 g N + 0.085 g of P per kg soil from NH_4NO_3 and K_2HPO_4)] applied to the soil taken from oil

storage site with an initial TPH of 14,000 mg kg^{-1}. After the 140 day operation, they found that the polluted soil amended with kitchen waste and the low-level nutrient (11:3.7 N:P ratio) achieved the highest total petroleum hydrocarbon degradation efficiency of up to 80 % during 70 days.

Abdulsalam et al., 2011 has conducted a study to compare biostimulation (i.e. 30.42 g of NPK fertilizer per kg soil and 5.6 g of KH2PO4 per 1.5 kg of soil to give 10:1 N:P ratio) and bioaugmentation (consortium of bacteria i.e. *Pseudomonas aeruginosa* and *Bacillus subtilis)*) for the remediation of soil contaminated with motor oil in Nigeria. The results showed that biodegradation was effective and that 66 % and 75 % of oil was removed using bioaugmentation and biostimulation respectively after ten weeks of experiment. Again this is a study where similar effects of biostimulation were seen to those observed in the current investigation.

Sabate et al., 2004 and Farahat et al., 2008 reported similar results with a residual fraction in soil after a bioremediation experiment and also demonstrated that the degradation pattern of organic chemicals in soil usually shows a rapidly degradation followed by a period of little or no change in concentration over time. However, soil amended with nutrients showed little more TPH reduction than the soil amended with water only during the whole period of incubation. In addition, the maximum degradation of TPH was observed at 40°C. The current study shows that biodegradation of the crude oil contaminated soil using biostimulation and bioaugmentation methods are effective and confirms that bioremediation of hydrocarbons in soil is feasible at both temperatures (25–40°C), in addition soil salinity as investigated at a lab scale. The oil-degrading microorganisms found in the polluted soils at the Nasser oilfield, can degrade hydrocarbons which suggest that the bacteria that found in the soil are adapted to the crude oil and salts polluted soil (i.e. within their environment) at the study site. Similar observations were reported by Balba et al 1998 who applied bioremediation to desert soil highly polluted with crude oil and of high salinity from Kuwait using three different bioremediation methods. The data show that an 82.5 % to 90.5 % reduction in the total petroleum hydrocarbons within a 12-month period, depending on the bioremediation method used. In the present study the data show that the highest degradation rates of the three crude oil contaminated soil investigated by biostimulation and bioaugmentation methods were obeserved in contaminated soil with low crude oil (0.87 % oil). The data show that 99.10 % of TPH was removed from soil with low crude oil content (0.87 %) comparing with those soil contaminated with 3.8 % and 7.2 % of crude oil. These

observations are in agreement with Salanitro et al., 1997 who applied bioremediation experiments to the soil contaminated with three concentrations of crude oil. They found that the highest degradation rate occurred in low crude oil contaminated soil, in which 76 % of TPH was removed after 4 months of incubation. From this study it is clear that by providing suitable environment conditions effective bioremediation of crude oil contaminated soil on the Nasser oilfield could be achieved and that it may also reduce the time required remediating contaminated soil with over 99 % degradation is possible within 240 days.

6.14 Microbiology analysis

Microbial content analysis methods depend on the efficient extraction of nucleic acids from the microorganisms of interest. There are reports of many different techniques to extract nucleic acids from soil microbial communities. These techniques extract nucleic acids directly or indirectly from microorganisms in soil. Microbes are the main degraders of petroleum hydrocarbons in contaminated ecosystem. The isolation of different oil degraders from soil using a crude oil as carbon and energy source serial enrichments was needed (Chikere et al., 2011 and Stoica et al., 2012). The overall aim of this section is to develop a consortium of microorganisms that can efficiently degrade hydrocarbon contaminates.

6.14.1 Montoring of microbiological content in soil

Soil usually contains large number of native or indigenous microorganisms that are able to degrade petroleum hydrocarbons. Microbial inhibition may occur in the presence of high salt concentration and heavy metals (Gogoi et al., 2003). In addition, hydrocarbon levels higher than 10 % are associated with varying degrees of inhibitory effects on soil microbes. The availability of microbe contents in all treatments units were monitored over the course of the experiments on soil taken from the treatments flask, this is carried out randomly from time to time using observation of colonies growth on agar plate methods and observation on microscope. Figure 6-16 shows significant bacterial populations on the NA agar plate and it clear from the image the colonies present in treatment unit. The colonies of bacteria growing on the NMS agar supplemented with 1 % crude oil are shown Figures 6-17A and 6-17B. These Figures shows the growth of the hydrocarbon degrading microbes on the NMS agar plate contains 1 % crude oil. The plate shows to contain very clear single colonies. Microscopic analysis showed the presence of hydrocarbon tolerant microorganisms as shown in Figure 6-18 concentrated in one of the liquid phases, most likely the aqueous phase.

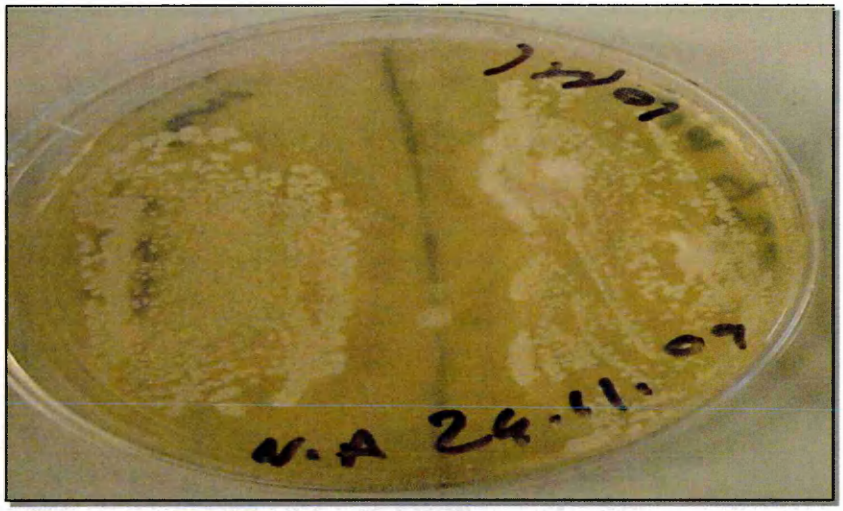


Figure 6-16 Colonies bacterial on NA agar plate

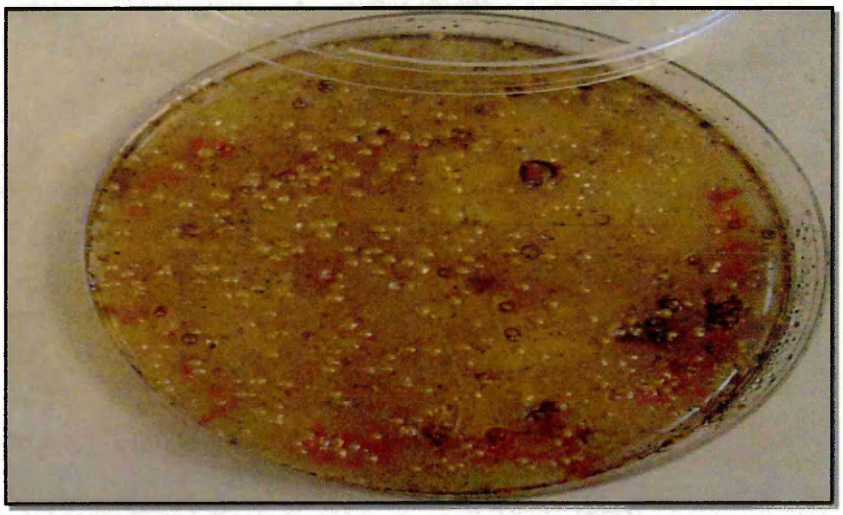


Figure 6-17A Colonies of hydrocarbon oxidizing bacterial on NMS agar plate supplemented with 1% crude oil

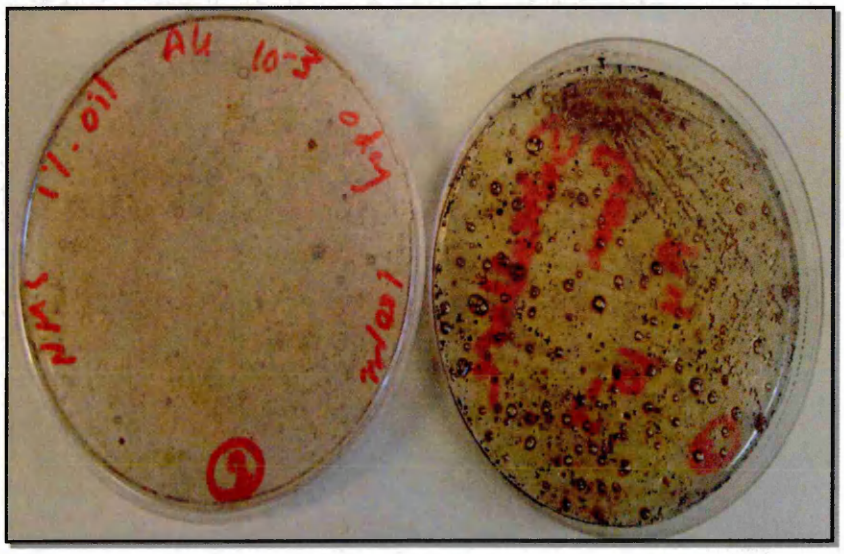


Figure 6-17B Colonies of hydrocarbon oxidizing bacterial on NMS agar plate supplemented with 1% crude oil

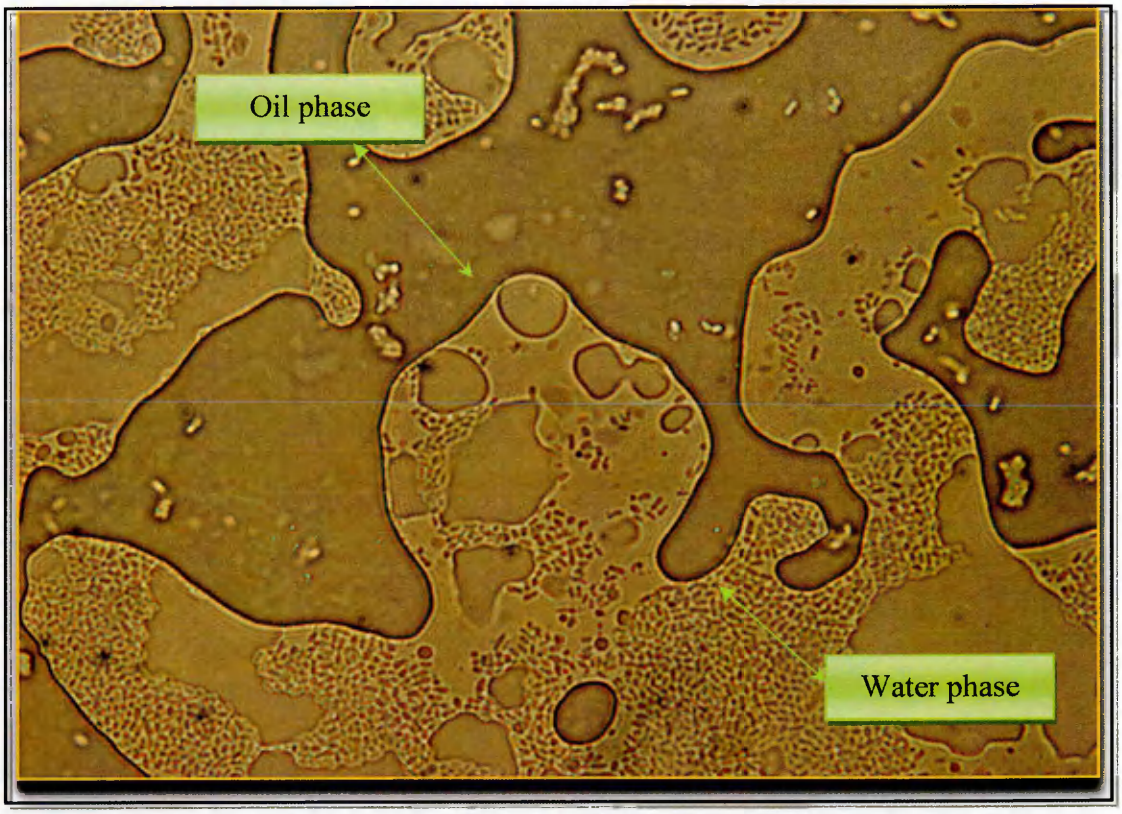


Figure 6-18A Microscope image of hydrocarbon degrading bacteria at water oil interface

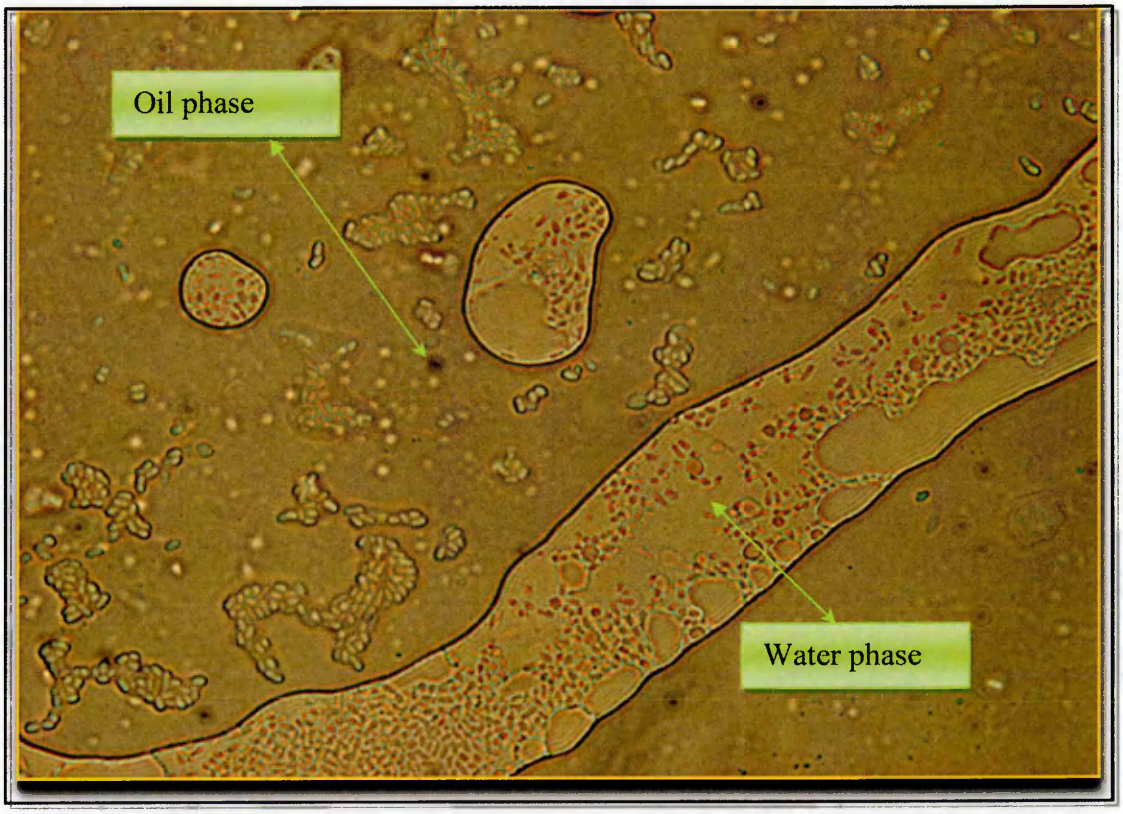


Figure 6-18B Microscope image of hydrocarbon degrading bacteria at water oil interface

6.14.2 Identification of aerobic bacteria from soil

Identification of bacteria is of great importance for the best understanding of the biodegradation process. In this study, selected single colonies were isolated by selective enrichment technique from randomly polluted soil treatment flasks. NMS and NA media were used in the enrichment technique supplemented with 1-3 % crude oil. A 0.1 ml of media was plated after appropriate dilution on NMS agar and incubated at 26°C; these isolates were then further streaked out on to NMS plates containing the appropriate concentration of oil to check purity. After 48 hour incubation, pure colonies were isolated by using a single colony isolation procedure. From these NMS plates six colonies were isolated that were different in colour, size and shape these were then subcultured on NA broth media as shown in Figure 6-19. The six isolated bacteria strains were identified via molecular biology, based on DNA extraction, genomic analysis of 16S rRNA genes and sequencing.

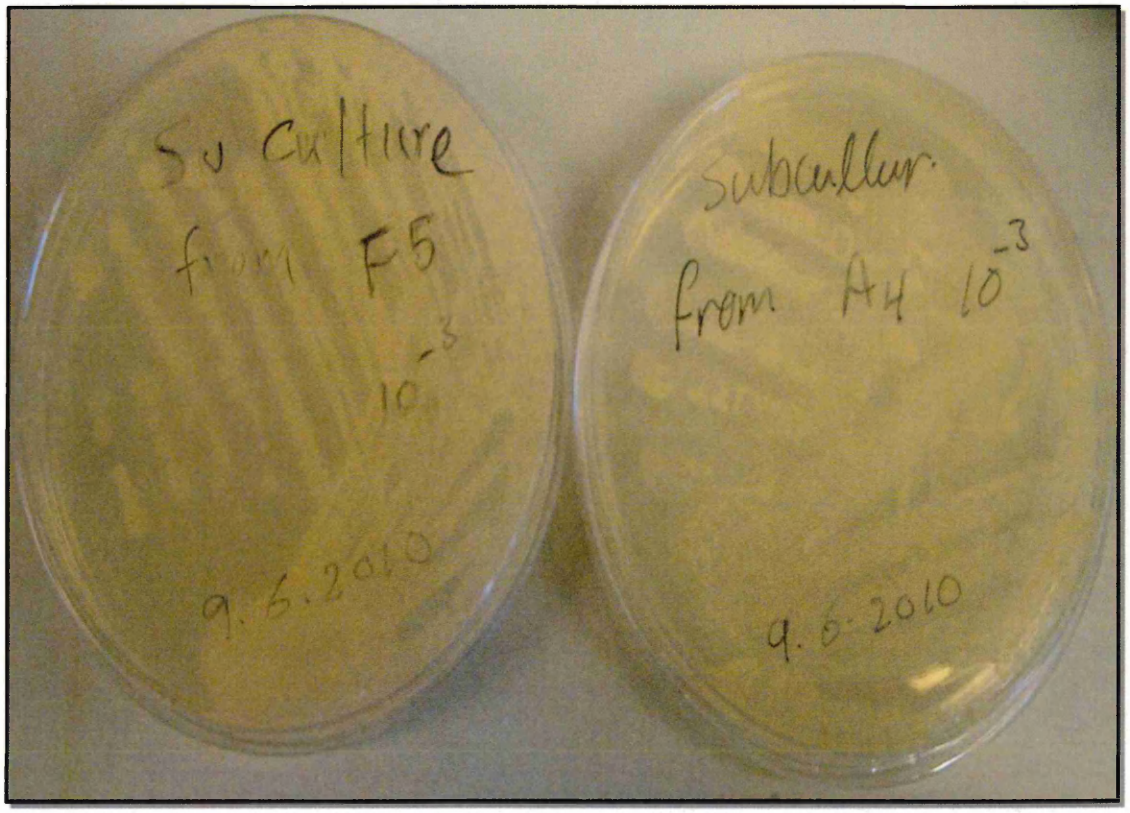


Figure 6-19 shows an example of isolated strains from an oil containing NMS agar plate subcultured on NA broth media

6.14.3 DNA extraction

Liquid cultures for DNA preparations were grown in nutrient broth medium after two rounds of purification to single colonies on NMS agar medium containing oil as the only carbon and energy source, as described in the preceding section. The six strains

for DNA preparations were chosen as strains that gave colonies different in colour, size and shape. The DNA preparations were performed using the QIAGEN Kit (QIAamp DNA mini kit) using manufactures protocols. This usually results in relatively pure DNA for subsequent molecular analysis. The yield and integrity of DNA obtained from soil samples was confirmed through electrophoresis in 1 % agarose gel stained with ethidium bromide and documented using a Bioimaging System as shown in Figure 6-20 (Lanes 1, 2, 3, 4, 5 and 6 are genomic DNA isolated from the samples).

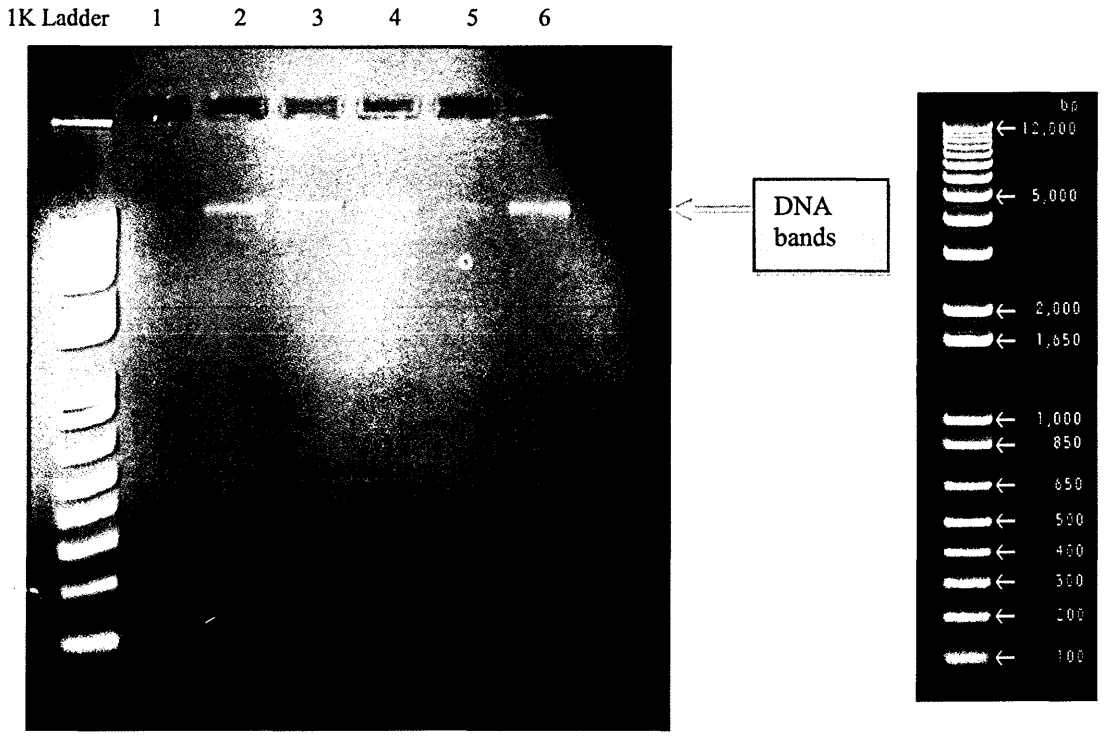


Figure 6- 20 Lanes 1, 2, 3, 4, 5 and 6 are genomic DNA extracted from isolated strains from the soil

6.14.4 16S rRNA gene-specific PCR

The DNA obtains from soil samples were then amplified using primers 16S-F (AGAGTTTGATCMTGGCTCAG) and 16S-R (TACGGYTACCTTGTTACGACTT) for the bacterial 16S rRNA gene (Lane 1991). The presence of PCR products was confirmed by electrophoresis analysing of 5 µl PCR product in 1 % (wt/v) agarose gel and staining with 0.5 mg/l ethidium bromide. Figure 6-21 shows the 16S rRNA PCR products before purification (lanes 2, 3, 4, 5, 6 and 7 are PCR products). In addition, lane 1 was the PCR reactions without DNA as negative controls (-ve.). It is clear from the image that PCR amplification of DNA was successfully.

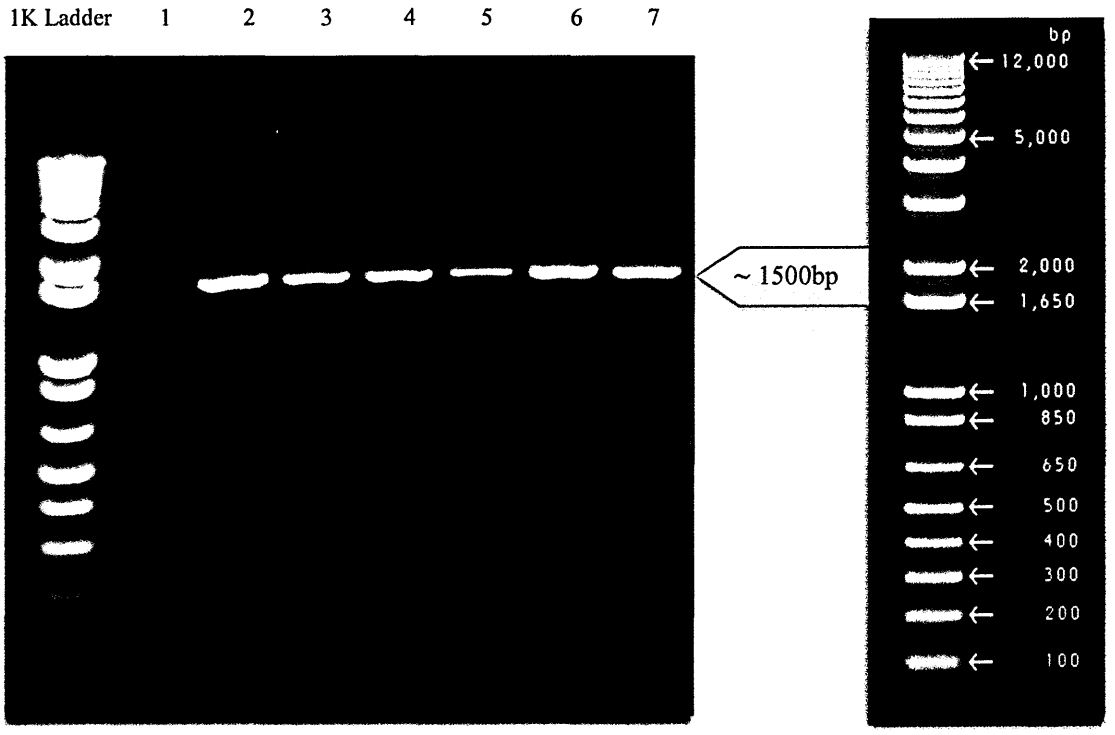


Figure 6- 21 16S rRNA PCR products before purification (lane 1 -ve control, lanes 2, 3, 4, 5, 6 and 7 are PCR products)

6.14.5 Purification of 16S rRNA gene PCR products and sequencing

In order to obtain a high quality PCR amplifiable DNA for sequencing, the 16S rRNA gene PCR products were purified using a QIAGEN MinElute PCR Purification Kit as described previously in section 6.10.4 of this chapter. The presence of 16S rRNA gene PCR products was confirmed by electrophoresis analysing of 5µl PCR product in 1% (wt/v) agarose gel and staining with 0.5 mg/l ethidium bromide. Figure 6-22 shows the different in intensity of 16S rRNA PCR products after purification (lanes 1, 2, 3, 4, 5 and 6 are PCR products). This intensity shows there is sufficient DNA to allow for sequencing.

6.14.6 Identification of strains

In order to identify the types of bacterial strain present in the soil samples the purified PCR products were sent to Eurofins MWG/ operon company, Germany for sequencing. The sequencing results of the samples of the large subunit 16S rRNA gene sequence were submitted to the Gen-Bank database (BLAST Assembled RefSeq Genomes) these results are presented in Table 6-6. The identification of each strain was based on the identity of their 16S rRNA gene sequence. The sequences of isolates 1 and 2 had 100 % identity with those of *Bacillus* sp., and *Microbacterium* sp., respectively. Isolates 3 and 4 had 99 % identity with *Bacillus simplex* and *Corynebacterium* stationis strain respectively. The 16S rRNA indicated that the strains

is closely related to *Bacillus* sp., *Microbacterium* sp., *Bacillus simplex and Corynebacterium stationis*. These bacteria have all been previously described in the literature as hydrocarbon degraders and/or associated with oilfield environments.

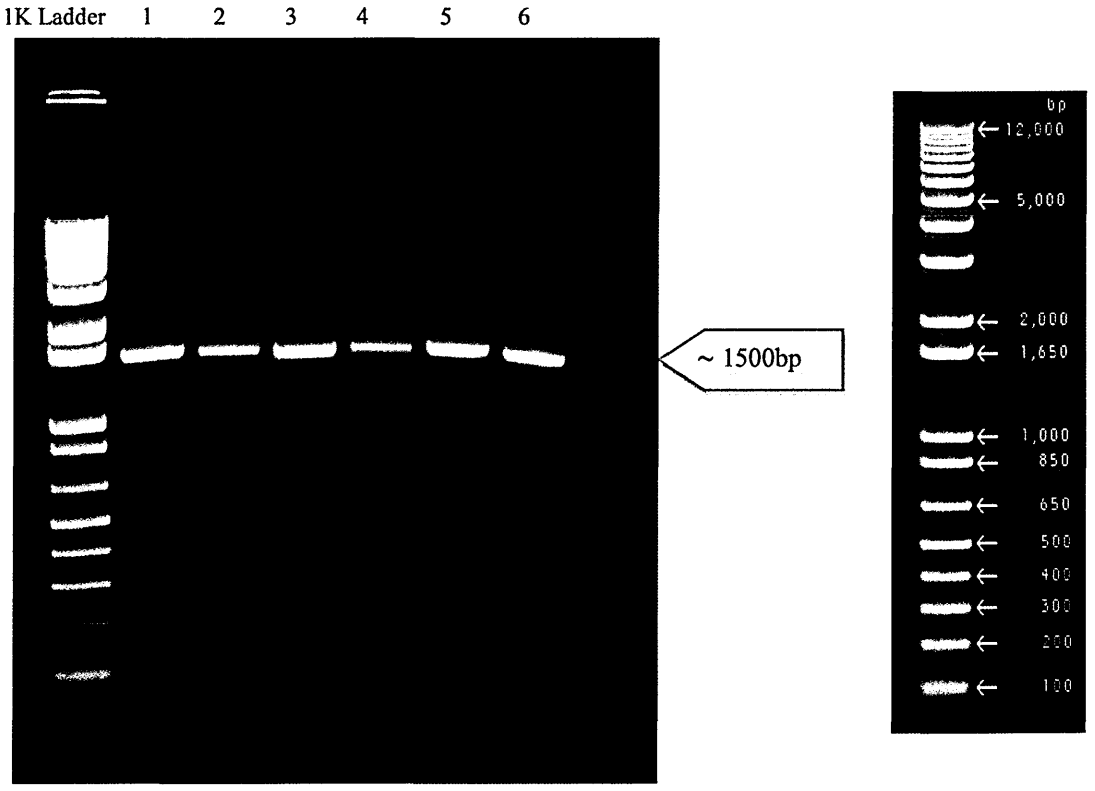


Figure 6- 22 16S rRNA PCR products after purification (lanes 1, 2, 3, 4, 5 and 6 are PCR products)

Table 6-6 Characteristics of isolates microbe from soil at the Nasser oilfield

Isolates	Gram stain	Accession No.	Closed strain	Clipped lenght	% Identity
1	Gram +	FR746080.1	*Bacillus* sp.	935	99 %
2	Gram +	FJ210845.1	*Microbacterium* sp.	976	99 %
3	Gram +	FJ210845.1	*Microbacterium* sp.	969	100 %
4	Gram +	FJ172668.1	*Corynebacterium*	429	99 %
5	Gram +	GU188928.1	*Bacillus* simples strain	941	99 %
6	Gram +	EF204409.1	*Microbacterium* sp.	975	100 %

Over the past decade, numerous species of bacterial strains have been reported as able to utiliz petroleum hydrocarbons from the environment, such as *Pseudomonas, Bacillus, Acinetobacter, Arthrobacter, Achromobacter, Corynebacterium, Nocardia, Flavobacterium, fluorescens, Mycobacterium, Micrococcus, Haemophilus, Aspergillus, Rhodococcus, Paenibacillus, Microbacterium, Trichoderma, Morteilla and Ralstonia* (Atlas 1981, Leahy 1990, Riser-Roberts 1998, Song et al, 2006, Joo et al., 2008, Haritash et al., 2009, De Carvalho et al., 2011, Das et al., 2011 and Onuoha et al, 2011). However, in this study some similar strains were found and isolated from the soil and identified as *Bacillus* sp., *Bacillus simplex* strain, *Microbacterium* sp and *Corynebacterium stationis* strain based solely on the 16S rRNA gene sequencing as shown in Table 6-6. Similar results were found in previous studies that were conducted on bioremediation of petroleum contaminated soil in other parts of the world.

Vasudevan et al., 2001 has isolated *Bacllus, Corynebacterium* and other strains from polluted soil collected from open field near petroleum refinery in Chennai City, India. These authors found that the addition of bacterial consortium in different amendments significantly enhanced the removal of oil from petroleum sludge of different treatment unit. Rahman et al., 2003, has investigated the distribution of biosurfactant production and crude oil degradation by bacteria in gasoline and diesel fuel polluted soil. Thirty two oil degrading bacteria were isolated including *Bacillus* sp., and *Corynebacterium* from different contaminated sites. It was concluded that the *Bacillus* sp. and *Corynebacterium* strains had the highest emulsification activity and crude oil degradation ability and were selected for the preparation of a mixed bacterial consortium oil degrading culture. Saadoun et al., 2008 who reported isolation of *Bacillus* and *Corynebacterium* from the crude oil polluted Jordan and Iraq desert soil samples and considered as the two organisms with major roles degradation of petroleum hydrocarbons. Similarly, Abu et al., 2008 studied the bioremediation of Niger Delta soil impacted with crude oil under laboratory conditions for 50 days period of bioremedation. The microorganisms isolated and identified in the study included, *Bacllus, Corynebacterium* other bacteria were also found identified namely, *Actinomyces, Arthrobacter, Brevibacterium, Pseudomonas*, and *Serratia* has the ability to degraded hydrocarbons. Moreover, Amund O.O. et al., 1987 and Onuoha et al., 2011 have isolated three bacterial from motor oil polluted soil and identified as *Corynebacterium, Bacillus* and *Pseudomonas* sp. They found that *Corynebacterium* species had the highest ability to degrade motor oil. Furthermore, Chaillan et al., 2004

has reported and proved that *Microbacterium* isolated from petroleum polluted soil at Indonesian has the potential for hydrocarbon degradation. Moreover, Makut et al., 2010 investigated the bacterial flora of soils contaminated with used oil in Keffi town, Nigeria. The species isolated *Bacillus* sp., and *Microbacterium* sp., were able to utilize used oil. They also isolated other microorganisms such as *Pseudomonas* sp., *Streptococcus* sp., *Escherichia coli, Staphylococcus* sp., *Klebsiella* sp., *Enterobacter aerogenes, Salmonella* sp., and *Micrococcus* sp. Numerous studies show that the *Bacillus* isolates from hydrocarbon contaminated sites have potential in the treatment of oil spills (Bento et al., 2005, Singh et al., 2008, Nkwelang et al., 2008 and Cerqueira et al., 2012). Hassan, et al., 2012 investigated the potential for enhancing the rate of crude oil bioremediation in crude oil contaminated soil with addition of organic materials as nutrient derived from local plants. They reported the isolation of *Microbacterium, Sphingopyxis* and *Bordetell petrii* from the crude oil contaminated soil collected from Al-Sabreia oilfield, Kuwait. They found that that those genes are able to enhanced crude oil degradation from soil.

Accordingly, to Joo et al., 2008 there are approximately 70 genera of known oil-degrading microorganisms, including *Bacillus* and *Microbacterium*. Savapornl et al., 2006 has isolated various bacterial strains from soil contaminated with lubricant oil collected around storage tank in Bangkok, Thailand. It was found that 3 of the strains showed high sequence similarity with 16S rRNA genes form *Bacillus, Microbacterium* and *Pseudomonas*. It was concluded that this bacteria had the ability to grow in mineral salt media containing lubricant oil used as sole carbon and energy source as well as has the ability to break down oil contained within sand.

Previous work has shown the microorganisms closely related to those isolated in the current study have the ability to oxidise hydrocarbons. Since the strains isolated here grew with oil as the only carbon source during two rounds of colony purification and subsequently in nutrient broth, they appear to be facultative oil-degraders that may be worthy of further study.

6.15 Conclusions

Crude oil released onto the soil, cause damage to the natural environment and ecosystems. However, when treated appropriately by optimizing the biodegradation potential of naturally occurring hydrocarbon degraders, the main part of hydrocarbon pollution can be reduced by biodegradation process. The most important conclusions which could be drawn from this chapter are summarized in the following points:-

- The present data show the presence of indigenous hydrocarbon degrading microorganisms in the Nasser oilfield soil.
- The eight months results obtained from the lab scale bioremediation experiments showed significant oil biodegradation results. Oil biodegradation was enhanced significantly by providing the soil with nutrients, moisture, and oxygen.
- The indigenous soil microorganisms were capable of biodegrading the oil hydrocarbons. Biodegradation of TPH in soil was observed in treatment units in both biostimulation and bioaugmentation methods.
- The results shows that TPH concentrations decreased by 73 -99 % over the course of 240 days of biodegradation experiments. The results show that the first 60 days, the presence of soil greatly enhanced TPH biodegradation, and the 60-2400 days soil slightly enhanced TPH biodegradation.
- The study shows there was residual crude oil in the soil after 240 days of investigation. These concentrations of TPH are more persistent and take longer to degrade. An experimental run of longer than 240 days period would be desired.
- The results of this study demonstrate that the microorganisms from the field site have the ability to degrade petroleum hydrocarbons that exist into the soil. They play an important role in the natural attenuation of the petroleum pollutants. Bacteria in the polluted soil can degrade hydrocarbons which suggest that the bacteria that fond in the soil are adapted to the crude oil and salts polluted soil at the Nasser oilfield.
- The degradation of crude-oil contaminated soils with biostimulation and bioaugmentation were found to accelerate the degradation of TPH in the three concentrations of crude oil contaminated soil used with no big difference between both techniques.

- The investigation has shown that hydrocarbon biodegradability and its fate in the environment strongly depends upon the type and concentration of hydrocarbons.
- Species identification using PCR- gel electrophoresis of 16S rDNA gene fragments revealed the presence of known petroleum hydrocarbon degrading microbes

- The study shows that despite the high soil salinity an acceptable biodegradation rate can be achieved. It is expected that the indigenous microorganisms in this environment are salt-adapted. However, further biodegradation and molecular studies are needed to decipher the catabolic genes resident in these microorganisms and their hydrocarbon specificities. Such analysis may assist in developing cost effective and efficient bioremediation protocol for oil polluted soil at the Nasser oilfield pit.

Chapter Seven
Conclusions and Future work

7.0 Conclusions

Exploration and production of crude oil and natural gas usually results in significant volumes of wastes from the facility of the oil industry such as drilling waste and associated water (i.e. FW and IW). When oil and gas are produced, they are brought to the surface along with this water as a comined produced fluid. Once the FW is brought to the surface and seperated from oil, this water is called PW.

PW is one of the most important sources of pollution associated with crude oil and natural gas production. At the Nasser oilfield, Libya, large volume (315,000 barrels per day) of oily PWs were generated and directly discharged to the soil surface into open environment causing an environmental problem to the ecosystem and could be a possible health risk for local people.

The identification of pollutants allows for development of reliable treatment design criteria to help ensure that effective and consistent treatment is achieved to meet guideline levels required for disposal, or other beneficial uses.

The most important conclusions which could be drawn from this study are summarized in the following points:-

- The satellite scenes were acquired within a period of 34 years (1972-2006) at the Nasser Oilfield during different pumping rates. The main conclusion for the work in the Nasser Oilfield producing water pit is the usefulness of a combination of different satellite data sources such as Landsat MSS, TM, ETM, SPOT, Aerial Photography, and any other available satellite data. The results shows that the fusion of optical remote sensing data can yield more information about pit clarity, expansion, and depth of the pit and the procedure to routinely monitor such phenomena with the aid of satellite data has provided a good overview of polluted areas, as well as more natural environmental events. The results show that the expansion and depth of the pit can be estimated over very large areas via satellite data. It can be able to answer such questions as how pit clarity, expansion, and depth has changed over time, where lake management activities might be most useful, and which lakes will be most subject to change in the future due to such factors as changes in water flow. Satellite records of pit water sizes give you a good indication of whether there is going to be a systematic or major problem in water supply. The study shows that satellite images aid in showing what cannot be measured or seen by other traditional

techniques where the changes have been measured accurately during the period from 1972 till 2006.

- An attempt has been made to investigate the compounds that contribute to the environmental impact of produced water from the Nasser Oilfield Libya.

- The measurements of the physical and chemicals properties of PW generated by the Nasser oilfield operation; the high saline produced water was considering the major carrier of cations and anions and impact on the surrounding environment, where these accumulated materials (cations and anions). Unfortunately, under influence of PW discharges from the Nasser Oilfield operation at the surface soil the changes in properties of soil are likely (i.e. permeability, porosity, hydro-conductivity) and may effect the organisms present in the soil which are considered as element of the ecosystem.

- Soil samples obtained from the disposal pit at the study site show variable impacts from the disposal of PW on the surface. This soil was contaminated by saline water over long term of disposed: so high concentration of anions and cations exist in the soil, especially sodium, magnesium, calcium, potassium, chloride and sulfate. These cause the poor physical condition of the soil and accordingly to the results the soil is considered as saline and sodic.

- It was observed that the soil was suffering from high concentration of salts. Where the analysis results of reference soil samples taken from 250 meter from the edge of the pit are uncontaminated and were employed to make a comparison with the results of polluted samples.

- The result of the soil samples shows that the extents of pollution or influenced in soil was extent up to 20-25 meters from the edge of the pit.

- The analytical technique including IR, GC–FID, GC–MS and SPME techniques used shows that the produced water samples were contaminated by TPH, PAHs and BTEX compounds and when produced water released onto the open environment these contaminated will cause serious damage to the ecosystem and it clear that PW discharge to the open environment contains varying concentration of TPH, PAHs and BTEX.

- The results indicated that highly contaminated PW at the Nasser Oilfield discharged directly without any sort of treatment or manipulation into the surface soil onto open environment and this is of environmental concern.

- The results shows that the PW is the main source of pollution, many constituents are carried by it, especially; salts and hydrocarbons where directly disposed to the environment, and it causes environmental impact.

- The results obtained showed that a substation amount of crude oil is being discharged with the PW, so that pollution prevention opportunities are going to be most effective when they are coordinated with production sector, where efficiency of separation could be increased by developing of separation performance and technique, in order to prohibit or at least decrease oil free phase discharging with PW.

- Results from this study indicate that SPME coupled to GC–FID is a precise method for reproducibly analyzing BTEX in PW.

- TPH and PAHs, concentrations of soil samples taken from the area around the Nasser oilfield disposal pit were quantified and the results showed elevated pollution with TPH and PAHs. This is real and evident that the detected TPH and PAHs in soil samples were shown to have originated from oily PW disposal which considered the main source of the contamination at the study site.

- A soil sample was definitely affected by the disposal of PW.

- It was observed that the soil was suffering from high pollution by hydrocarbons and this is an expected result due to continuous accumulation of crude oil carried by PW.

- ESI-MS has been shown powerful technique for the identification of oilfield chemicals in soil and PW.

- SPE followed by ES-MS and HPLC coupled together has been proved very useful in the characterization of an important class of oilfield chemicals in soil and PW.

- LC coupled with ESI -MS method shows it is possible to separate OFCs peaks from other interfering peaks.

- Benzalkonium quaternary ammonium salts were successfully measured using SPE followed by ESI-MS and LC-ESI-MS in soil and PW samples collected from discharged pit at the Nasser oilfield, Libya. The residual OFCs that have been detected in PW samples need to be tested for toxicity (Acute and chronic toxicity).

- Crude oil released onto the soil, cause damage to the natural environment and ecosystem. However, when treated appropriately by optimizing the biodegradation potential of natural occurring hydrocarbon degraders, the main part of hydrocarbon pollution can be reduced by biodegradation process.

- Bioremediation is a promising technology for the treatment of a wide range of contaminants in soil.

- Bioremediation is cost-effective, particularly for dealing with petroleum hydrocarbon contamination, and can be easily integrated with other remedial technologies. The present study little difference was observed in soil amended with nutrients compared with soil amended with water only, from which it can be concluded that the microbes present within soil may be using the nutrients already available in the soil and that the availability of such nutrients is not a major limiting factor on the progress of hydrocarbon oxidation.

- Bioremediation is site-specific, and laboratory studies are therefore highly recommended before full-scale remediation is considered.

- The present data show the presence of indigenous hydrocarbon degrading microorganisms in the Nasser oilfield soil.

- The eight months results obtained from the lab scale bioremediation experiments showed almost complete oil biodegradation. Oil biodegradation was enhanced significantly by providing the soil with nutrients, moisture, and oxygen.

- The indigenous soil microorganisms were capable of biodegrading the oil hydrocarbons. Biodegradation of TPH in soil was observed in treatment units in both biostimulation and bioaugmentation methods.

- The results shows that TPH concentrations decreased by 73 -99 % over the course of 240 days of biodegradation experiments. The results show that the first

60 days, the presence of soil greatly enhanced TPH biodegradation, and from 60 days to 240 days soil shows slightly enhanced TPH biodegradation.

- The study shows there was residual crude oil in the soil after 240 days of investigation. After 240 days TPH is still present. These concentrations of TPH are more persistent and take longer to degrade. An experimental run of longer than 240 days period would be desired.

- The study show that the bacteria isolated from polluted soil of the Nasser oilfield has the ability to growth on the heavy oil and used as carbon and energy source.

- The results of this study demonstrate that the microorganisms from the field site have the ability to degrade petroleum hydrocarbons that exist into the soil. They play an important role in the natural attenuation of the petroleum pollutants. Bacteria in the polluted soil can degrade hydrocarbons which suggest that the bacteria that found in the soil are adapted to the crude oil and salts polluted soil at the Nasser oilfield.

- Species identification using PCR- gel electrophoresis of 16S rDNA gene fragments revealed the presence of known petroleum hydrocarbon degrading microbes

- The results of a lab scale study of bioremediation of crude oil-contaminated soils from the Nasser oilfields in Libya using biostimulation and bioaugmentation are reported.

- The degradation of crude-oil contaminated soils with biostimulation and bioaugmentation were found to accelerate the degradation of TPH in the three concentrations of crude oil contaminated soil used with small difference between both techniques. Therefore, biostimulation and bioaugmentation are recommended for bioremediating oil contaminated soil at the Nasser oilfield.

- From the three different concentrations of crude oil contaminated soil samples used the highest degradability were detected on low (0.87 %) and medium (3.8%) TPH concentrations after 240 days of incubation which 99.10 % and 98.12 % of TPH were removed from soil respectively.

- The study shows that despite the high soil salinity an acceptable biodegradation rate can be achieved. It is expected that the indigenous microorganisms in this

environment are salt-adapted. However, further biodegradation and molecular studies are needed to decipher the catabolic genes resident in these microorganisms and their hydrocarbon specificities. Such analysis may assist in developing cost effective and efficient bioremediation protocol for oil polluted soil at the Nasser oilfield pit.

- The study has shown that remediation by enhanced natural attenuation is effective in the clean up of polluted site in the Nasser oilfield as investigated at the lab scale.

7.1 Future work

Bioremediation of oilfield might be accomplished using the indigenous petroleum bacteria degrading by enhancing their activity. Naturally, great care should be taken when results of lab scale experiments are extrapolated to field situation because biodegradation studies in laboratory flask do not reveal field conditions with a great accuracy. Field trials must be performed to support conclusion drawn from lab experiments

- Screening of indigenous microorganisms with high TPH degrading ability should be investigated.
- Each of the isolated microbes from the studied soil should test for their ability to degrade crude oil separately in case of applying bioaugmentation.
- Determine the key parameters in order to perform pilot bioremediation experiments of mixed hydrocarbons on larger scale.
- Define limiting factor on rate of bioremediation to assess the best biological soil parameters to monitor TPH degradation in soil.
- Optimize bioremediation process (i.e. addition N, P, other trace elements and availability of microbes) further too completely degrade target contaminants.
- Molecular microbiology (i.e. DNA and 16S rRNA gene sequence) and chemicals analysis of optimised system.

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9.0 Poster Presentation

Salem Abdalla Omar[1,2], Tom J Smith[2] and Malcolm R. Clench[2]. "Analysis of Oilfield Chemicals in Soil and Produced Water by Using ESI/MS/MS and LC/MS/MS Techniques" at the 18 th (IMSC 2009) International Mass Spectrometry Conference held in Bremen on 30 August to 4th September 2009, **Germany.**

Salem Abdalla Omar[1,2], Tom J Smith[2] and Malcolm R. Clench[2]. "The Environmental Impact of Oilfield Formation Water on Soil at the Nasser oilfield, Libya" at the Biomedical Research Poster day held on 16th December 2009 - 3rd Price Award, at **Sheffield Hallam University, UK.**

Salem Abdalla Omar[1,2], Tom J Smith[2] and Malcolm R. Clench[2]. Determination of PAHs in the Oilfield Produced Water at the Nasser oilfield, Libya by Using Gas Chromatography Mass Spectrometry [GC-MS] at the 58th (ASMS) Conference on Mass Spectrometry and Allied Topics held in Salt Lake City, Utah on 23-27 May 2010, **USA.**

Salem Abdalla Omar[1,2], Tom J Smith[2] and Malcolm R. Clench[2]. "Analysis of Oilfield Chemicals in Soil and Produced Water by Using ESI/MS/MS and LC/MS/MS Techniques" at the 5th (EMS2010) National Meeting on Environmental Mass Spectrometry held on 17th March 24/ 2010 at **Chester University, UK.**

Salem Abdalla Omar[1,2], Tom J Smith[2] and Malcolm R. Clench[2]. "Analysis of Oilfield Chemicals in Soil and Produced Water by Using ESI/MS/MS and LC/MS/MS Techniques" at the Faculty of Health and Wellbeing Research Day held on 17th June 2010 at **Sheffield Hallam University, UK.**

Salem Abdalla Omar[1,2], Tom J Smith[2] and Malcolm R. Clench[2]. Determination of PAHs in the Oilfield Produced Water at the Nasser oilfield, Libya by Using Gas Chromatography Mass Spectrometry [GC-MS] at the Faculty of Health and Wellbeing Research Day held on 17th June 2010 at **Sheffield Hallam University, UK.**

Salem Abdalla Omar[1,2], Tom J Smith[2] and Malcolm R. Clench[2]. Bioremediation of Petroleum Hydrocarbons Contaminated Soil at the Nasser oilfield, Libya at the Faculty of Health and Wellbeing Research Day held on 30th June 2011 at **Sheffield Hallam University, UK.**

Appendix A

Chemical and physical properties of produced water

Appendix (A) Chemical and physical properties of produced water collected from the Nasser Oilfield, Libya

		Sample Code						
Parameter	**Unit**	**TA**	**Pump**	**SPT1**	**SPT2**	**SPT3**	**SPT4**	**WW**
Physical properties								
pH 24.4°C		6.90	6.67	7.30	7.41	7.46	7.44	7.42
Conductivity µS/cm@ 25°C		37057	38066	196977	189292	187867	194313	6675
Receptivity ohm/m@25°C		0.2699	0.2627	0.0508	0.0528	0.0532	0.0515	1.4981
TDS @ 180°C	mg/l	29580	25407	126065	121150	120235	124360	4500
TDS Calculated	mg/l	28049	24285	120249	117329	116384	119535	4279
Chemical properties								
Total Hardness as $CaCO_3$	mg/l	5330	6000	23600	23200	28600	29400	1520
Ca Hardness as $CaCO_3$	mg/l	3370	4400	16800	16600	20400	21200	740
Mg Hardness as $CaCO_3$	mg/l	1960	1600	6800	6600	8200	8200	780
Methyl Orange Alk. as $CaCO_3$	mg/l	160	126	100	110	90	100	170
Salinity as NaCl	mg/l	26614	23899	119926	117128	116282	118705	3766
Sodium	mg/l	8200	6650	36000	3500	32500	33250	860
Potassium	mg/l	280	320	1500	1500	1270	1270	50
Calcium	mg/l	1348	1760	6720	6640	8160	8480	296
Magnesium	mg/l	476	389	1652	1604	1993	1993	190
Total Cations	mg/l	10304	9119	45872	44744	43926	44993	1396
Chloride	mg/l	13614	13862	71155	69701	69148	69800	1540
Sulphate	mg/l	3983	1150	3100	2750	3200	4620	1236
Bicarbonate	mg/l	148	154	122	134	110	122	107
Carbonate	mg/l	0	0	0	0	0	0	0
Total Anions	mg/l	17745	15166	74377	72585	72458	74542	2883

Appendix B

Chemical and physical properties of soil

Appendix (B) Chemical and physical properties of soil from the Nasser Oilfield, Libya

		Sample Code						
Parameter	**unit**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**B1**
Physical properties								
pH 24.4°C		7.23	7.92	7.11	7.19	7.32	7.42	7.92
Conductivity µS/cm@ 25°C		88543	86826	62434	32800	39872	50036	73322
Receptivity ohm/m@25°C		0.1129	0.1152	0.1602	0.3049	0.2508	0.1999	0.1364
TDS @ 180°C	mg/l	57525	54700	39640	21300	25840	29960	43412
TDS Calculated	mg/l	56878	52416	38190	20454	24532	28798	43402
Chemical properties								
Total Hardness as $CaCO_3$	mg/l	11520	11200	8600	6240	7700	6870	10000
Ca Hardness as $CaCO_3$	mg/l	5840	6800	6240	5100	5490	4850	6400
Mg Hardness as $CaCO_3$	mg/l	5680	4400	2360	1140	2210	2020	3600
Methyl Orange Alk. as $CaCO_3$	mg/l	50	40	120	112	86	80	40
Salinity as NaCl	mg/l	56073	50514	36774	18380	23136	27956	39922
Sodium	mg/l	17000	15000	10800	4760	5800	8000	11500
Potassium	mg/l	360	580	280	200	230	250	720
Calcium	mg/l	2336	2720	2496	2040	2196	1940	2560
Magnesium	mg/l	1380	1070	574	278	538	490	864
Total Cations	mg/l	21076	19370	14150	7278	8764	10680	15644
Chloride	mg/l	31693	27398	20376	8740	11708	15694	19310
Sulphate	mg/l	4048	5600	3518	4300	3956	2326	8400
Bicarbonate	mg/l	61	48	146	136	104	98	48
Carbonate	mg/l	0	0	0	0	0	0	0
Total Anions	mg/l	35802	33046	24040	13176	15768	18118	25758

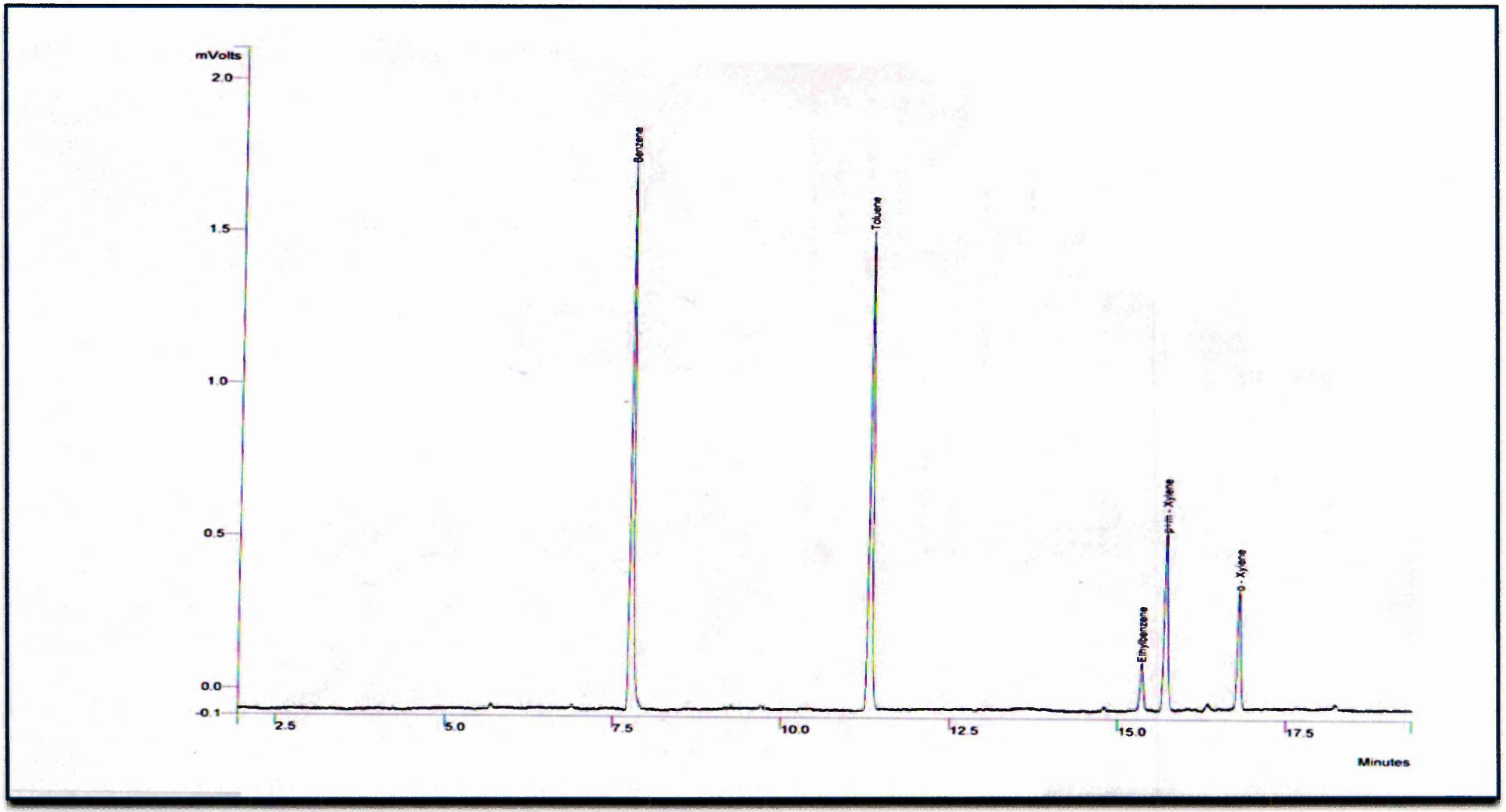
Appendix (B) Chemical and physical properties of soil from the Nasser Oilfield, Libya

		Sample Code						
Parameter	**unit**	**B2**	**B3**	**B4**	**B5**	**B6**	**C1**	**C2**
Physical properties								
pH 24.4°C		6.06	7.24	7.00	7.38	6.65	7.47	7.60
Conductivity µS /cm@ 25°C		161080	51586	53400	79746	110000	50424	31868
Receptivity ohm/m@25°C		0.0621	0.1938	0.1873	0.1254	0.0909	0.1979	0.3138
TDS @ 180°C	mg/l	105240	32900	33300	50240	72018	32840	20716
TDS Calculated	mg/l	103794	31804	33880	47836	68444	32304	20330
Chemical properties								
Total Hardness as $CaCO_3$	mg/l	2000	10820	9100	11200	7260	9510	9360
Ca Hardness as $CaCO_3$	mg/l	10240	6120	6300	7600	5000	7050	4900
Mg Hardness as $CaCO_3$	mg/l	9760	4700	2800	3600	2260	2460	4500
Methyl Orange Alk. as $CaCO_3$	mg/l	70	120	120	100	112	130	140
Salinity as NaCl	mg/l	104960	31742	31638	44468	66894	31584	18936
Sodium	mg/l	32000	7600	8600	13000	23200	8200	3500
Potassium	mg/l	640	330	336	360	400	320	190
Calcium	mg/l	4096	2448	2520	3040	2000	2820	1960
Magnesium	mg/l	2370	1142	680	874	550	598	1094
Total Cations	mg/l	39106	11520	12136	17274	26150	11938	6744
Chloride	mg/l	62722	18188	16798	22440	38358	18086	8884
Sulphate	mg/l	1880	1950	4800	8000	3800	2122	4532
Bicarbonate	mg/l	86	146	146	122	136	158	170
Carbonate	mg/l	0	0	0	0	0	0	0
Total Anions	mg/l	64688	20284	21744	30562	42294	20366	13586

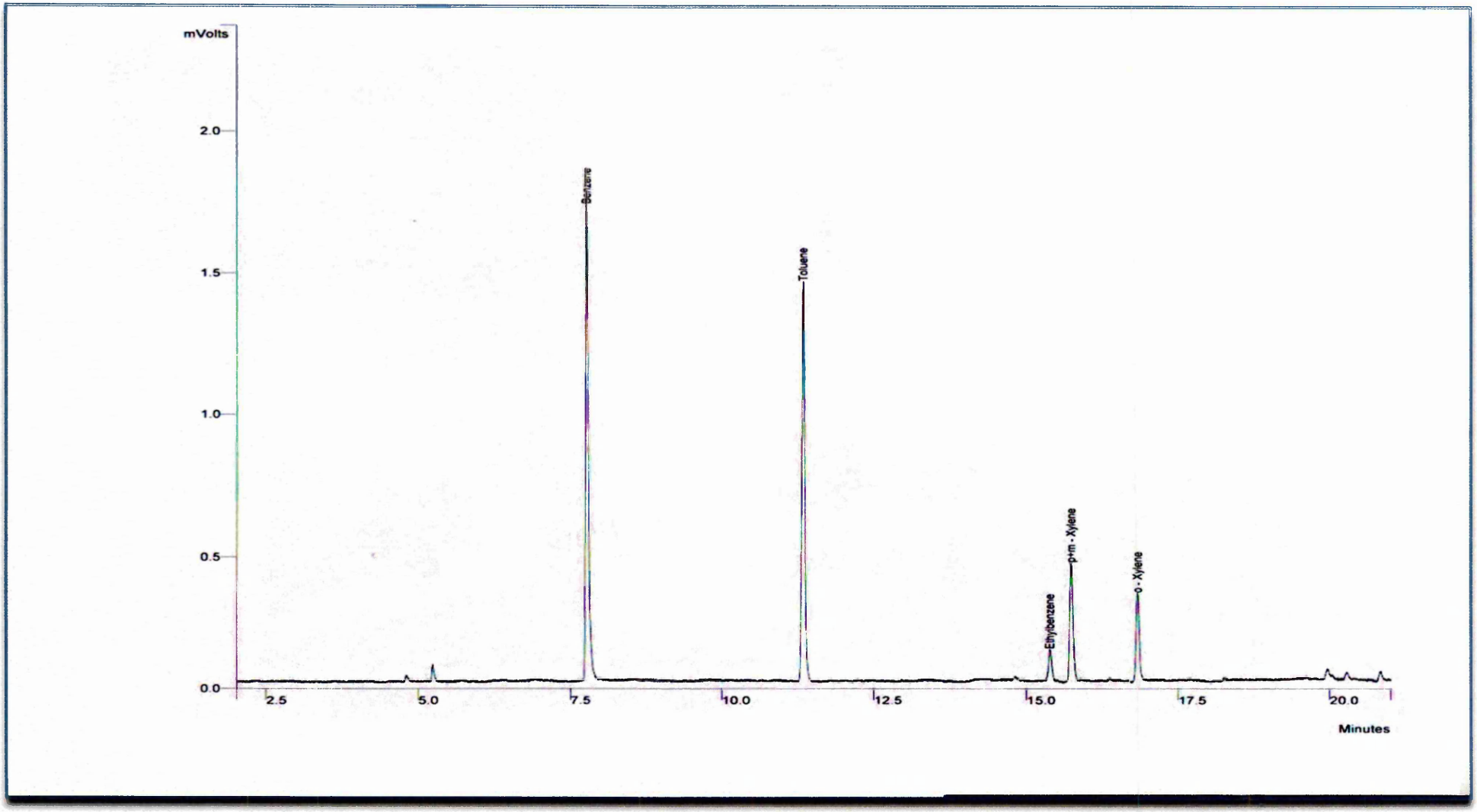
Appendix (B) Chemical and physical properties of soil from the Nasser Oilfield, Libya

		Sample Code				
Parameter	**unit**	**C3**	**C4**	**C5**	**C6**	**Ref.S**
Physical properties						
pH 24.4°C		6.62	6.85	7.48	6.61	7.27
Conductivity µS /cm@ 25°C		91586	46000	111484	89824	2346
Receptivity ohm/m@25°C		0.1092	0.2174	0.0897	0.1113	4.26
TDS @ 180°C	mg/l	59532	27920	71350	58244	1535
TDS Calculated	mg/l	58234	285 22	68797	57090	1478
Chemical properties						
Total Hardness as $CaCO_3$	mg/l	21720	7100	14000	14100	560
Ca Hardness as $CaCO_3$	mg/l	11210	5200	10000	8350	380
Mg Hardness as $CaCO_3$	mg/l	10510	1900	4000	5750	180
Methyl Orange Alk. as $CaCO_3$	mg/l	110	152	110	150	60
Salinity as NaCl	mg/l	54678	26902	67678	56826	1281
Sodium	mg/l	12400	7600	20250	16000	280
Potassium	mg/l	580	250	680	440	18
Calcium	mg/l	4484	2080	4000	3340	152
Magnesium	mg/l	2554	462	972	1398	44
Total Cations	mg/l	20018	10392	25902	21178	494
Chloride	mg/l	26506	13610	39211	33008	551
Sulphate	mg/l	11576	4400	3550	2720	360
Bicarbonate	mg/l	134	120	134	184	73
Carbonate	mg/l	0	0	0	0	0
Total Anions	mg/l	38216	18130	42895	35912	984

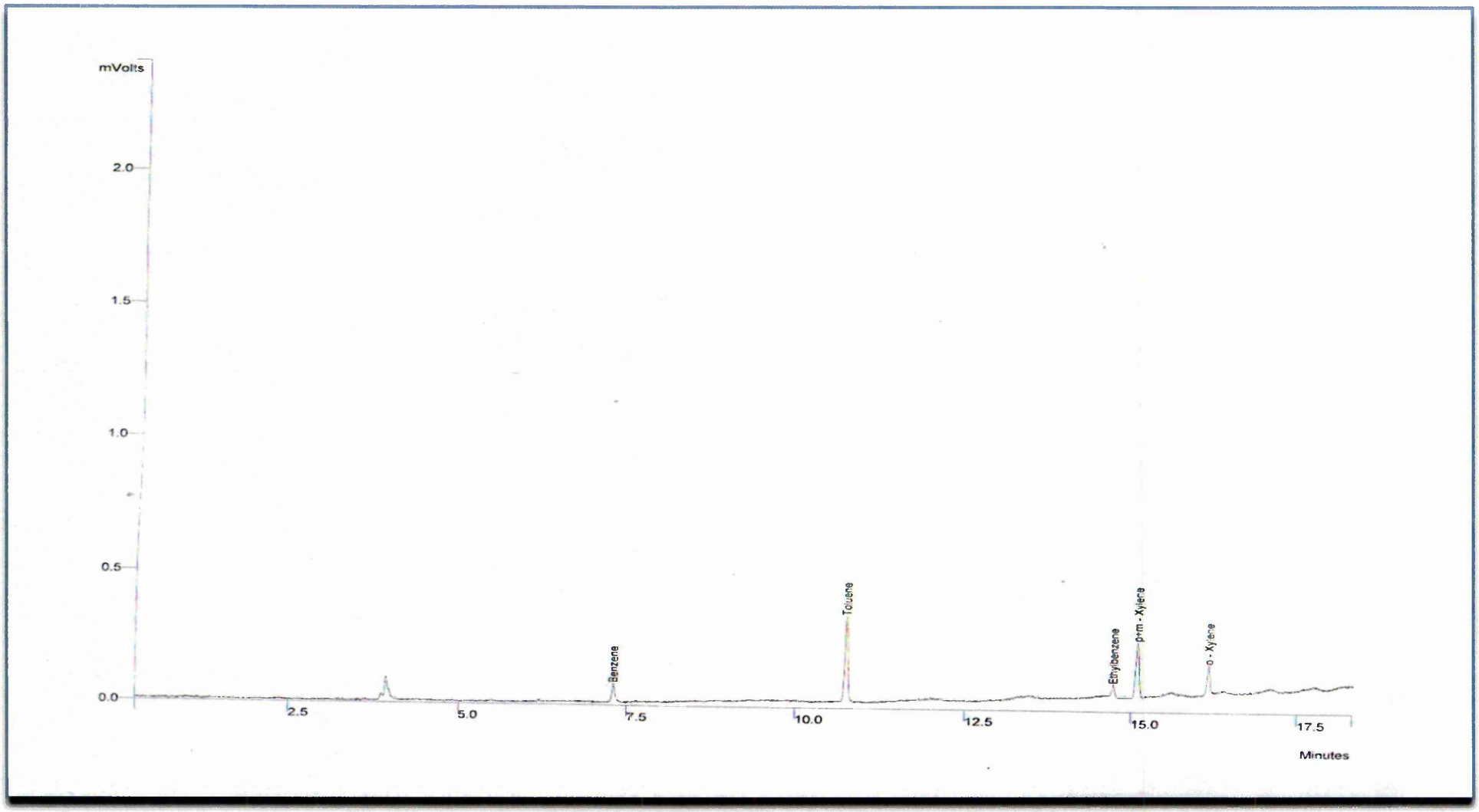
Appendix C
GC-FID chromatogram of BTEX compounds extracted from PW sample



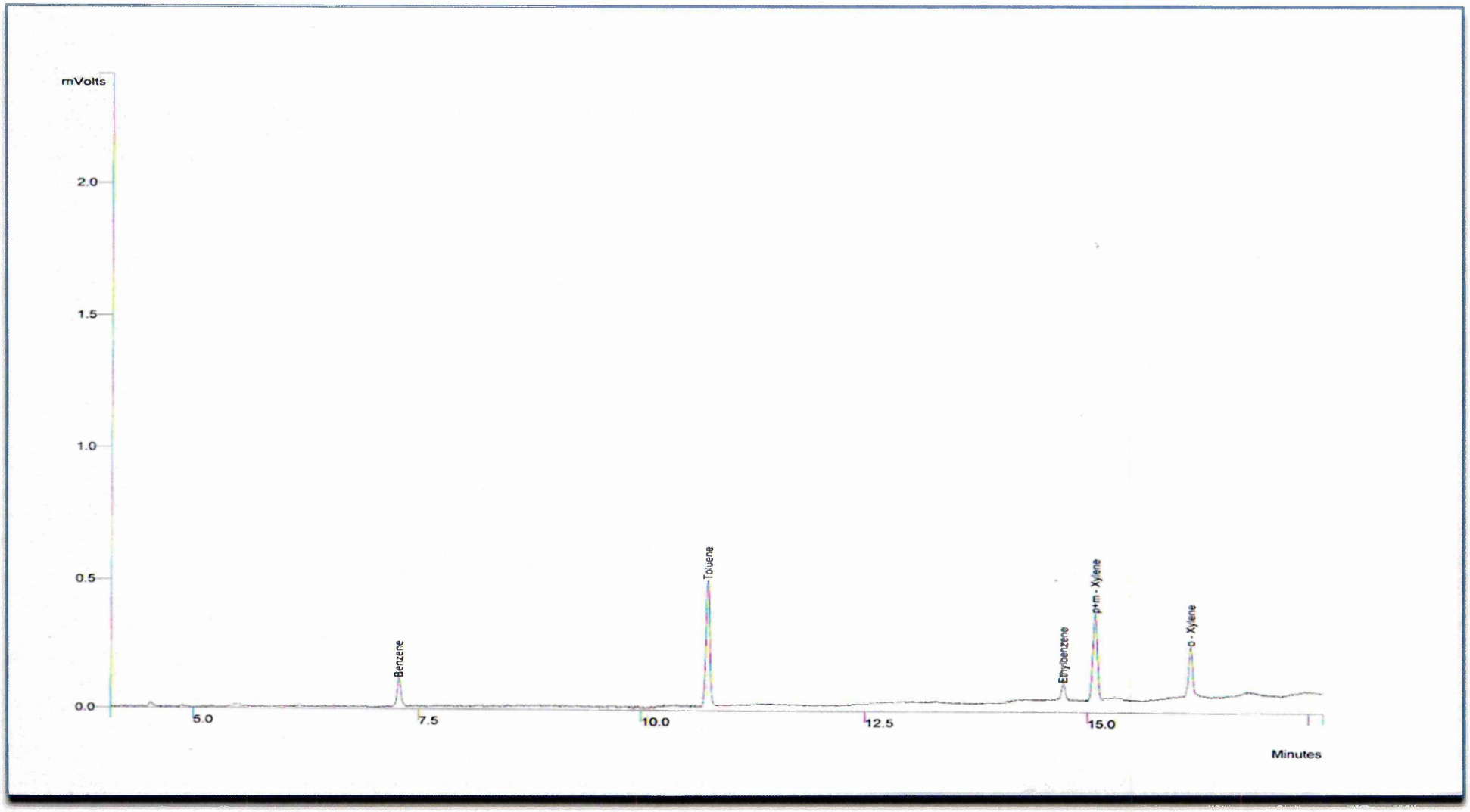
Appendix C1 GC-FID chromatogram of BTEX compounds extracted from produced water sample at settled tank (TA)



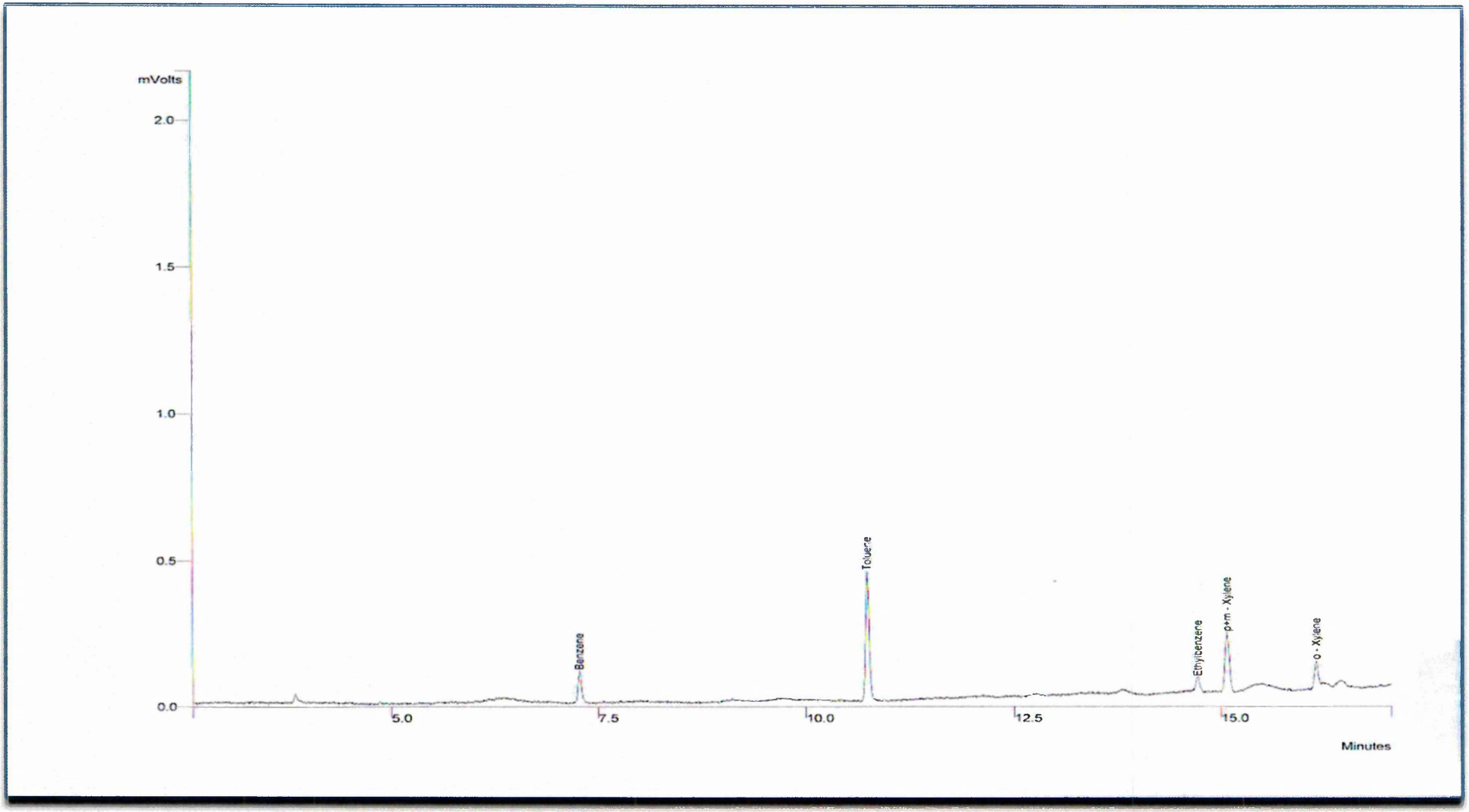
Appendix C2 GC-FID chromatogram of BTEX compounds extracted from produced water sample at pump station (Pump)



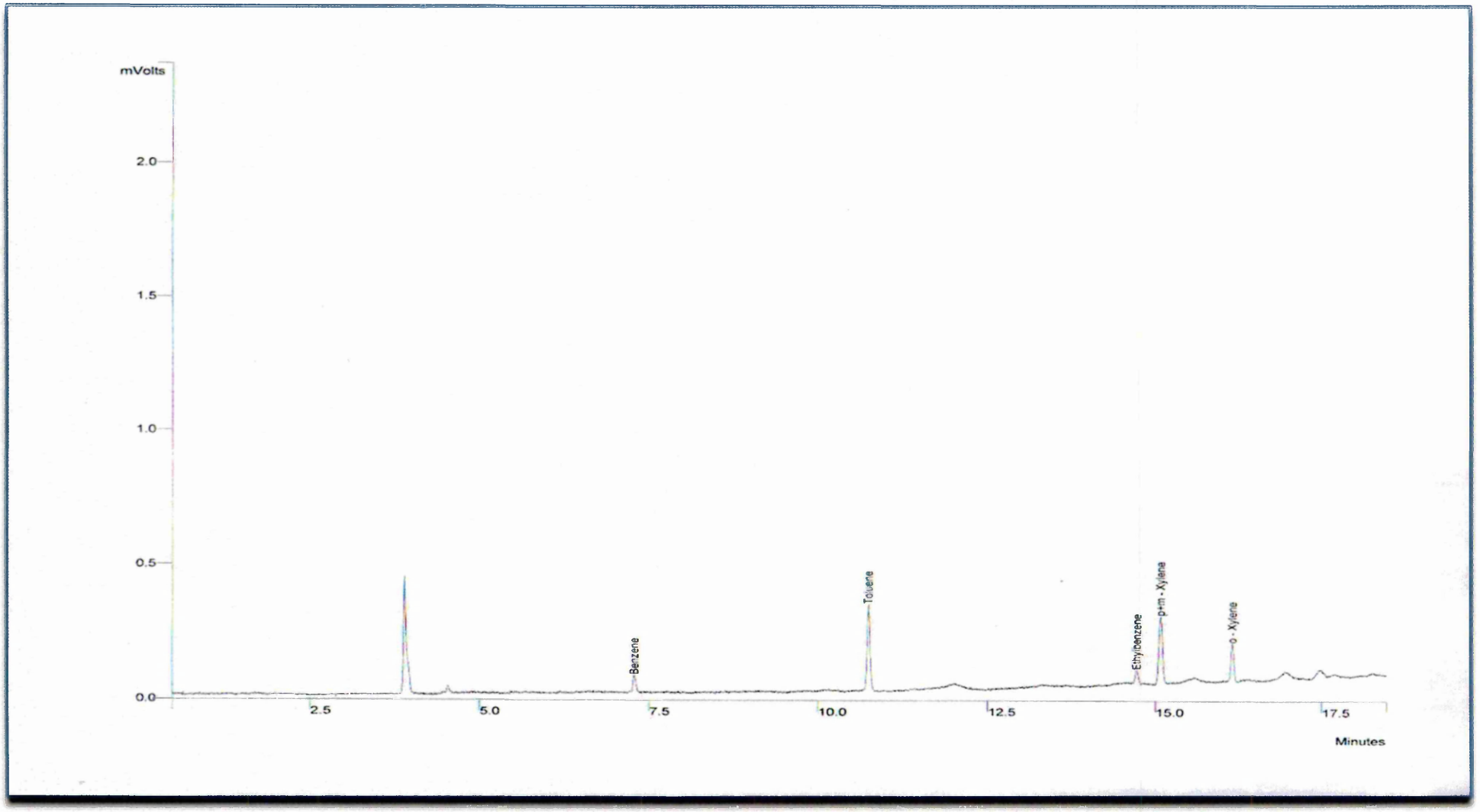
Appendix C3 GC-FID chromatogram of BTEX compounds extracted from produced water sample at pit location (Spt1)



Appendix C4 GC-FID chromatogram of BTEX compounds extracted from produced water sample at pit location (Spt2)

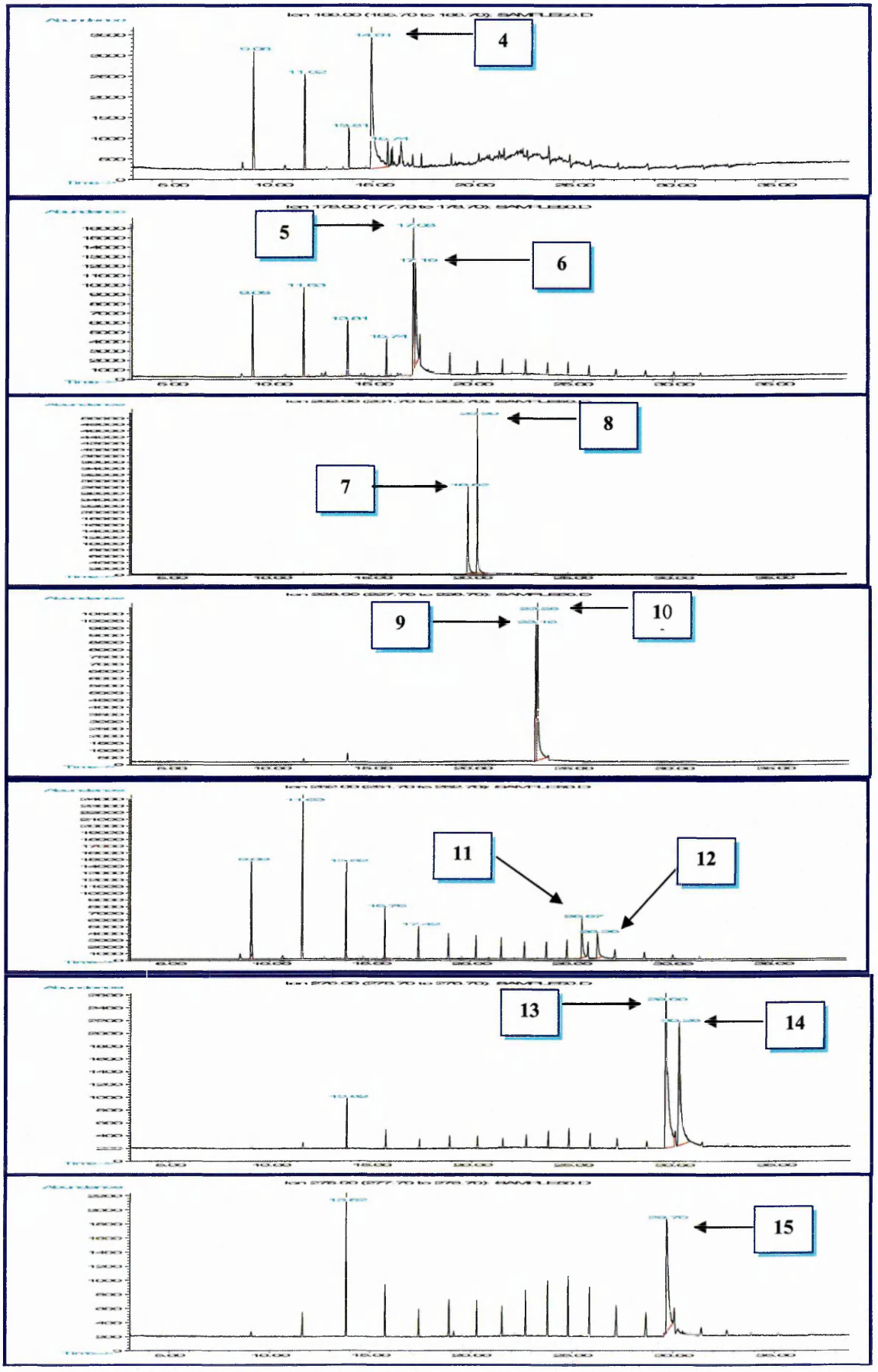


Appendix C5 GC-FID chromatogram of BTEX compounds extracted from produced water sample at pit location (Spt3)

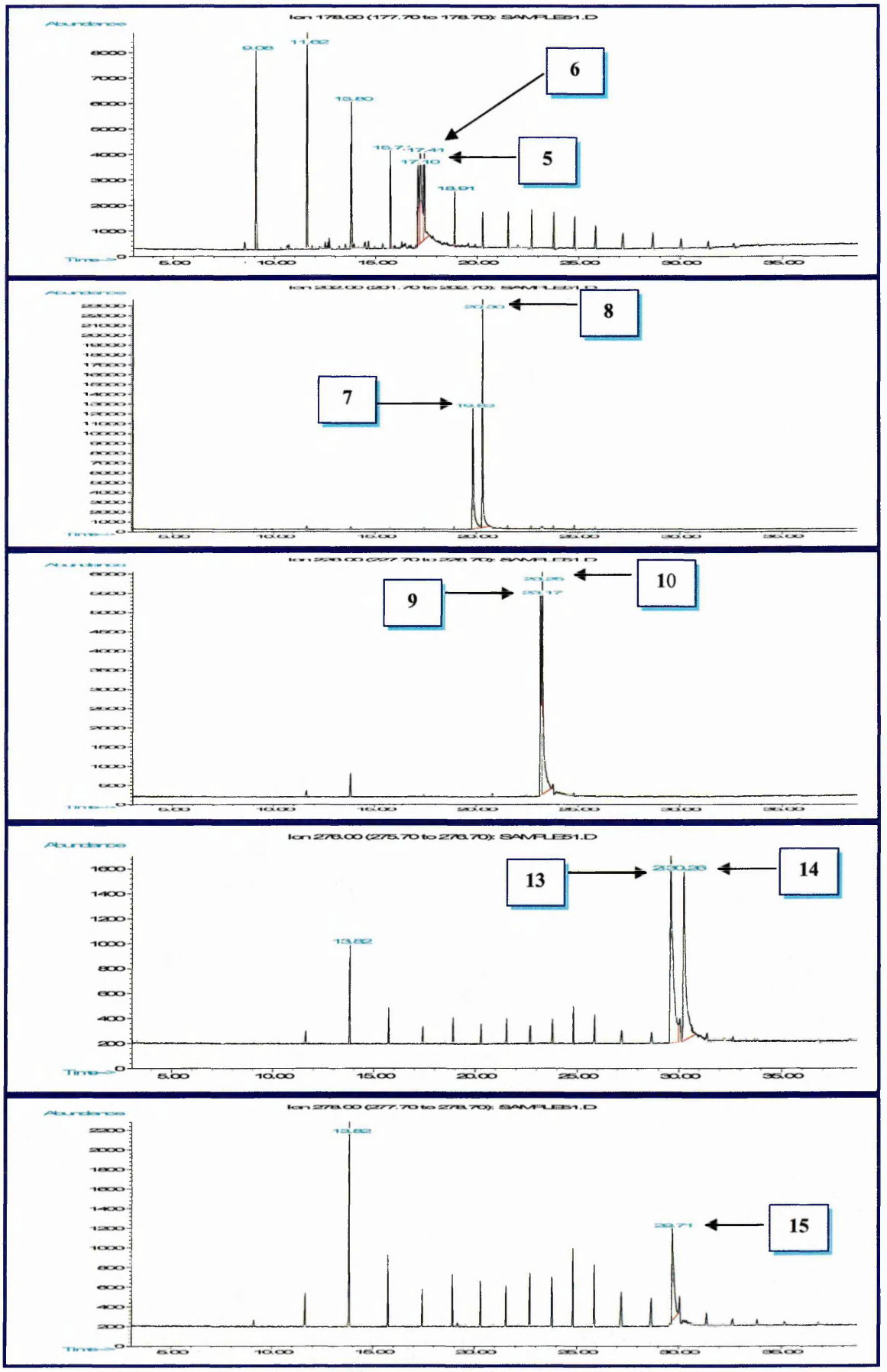


Appendix C6 GC-FID chromatogram of BTEX compounds extracted from produced water sample at pit location (Spt4)

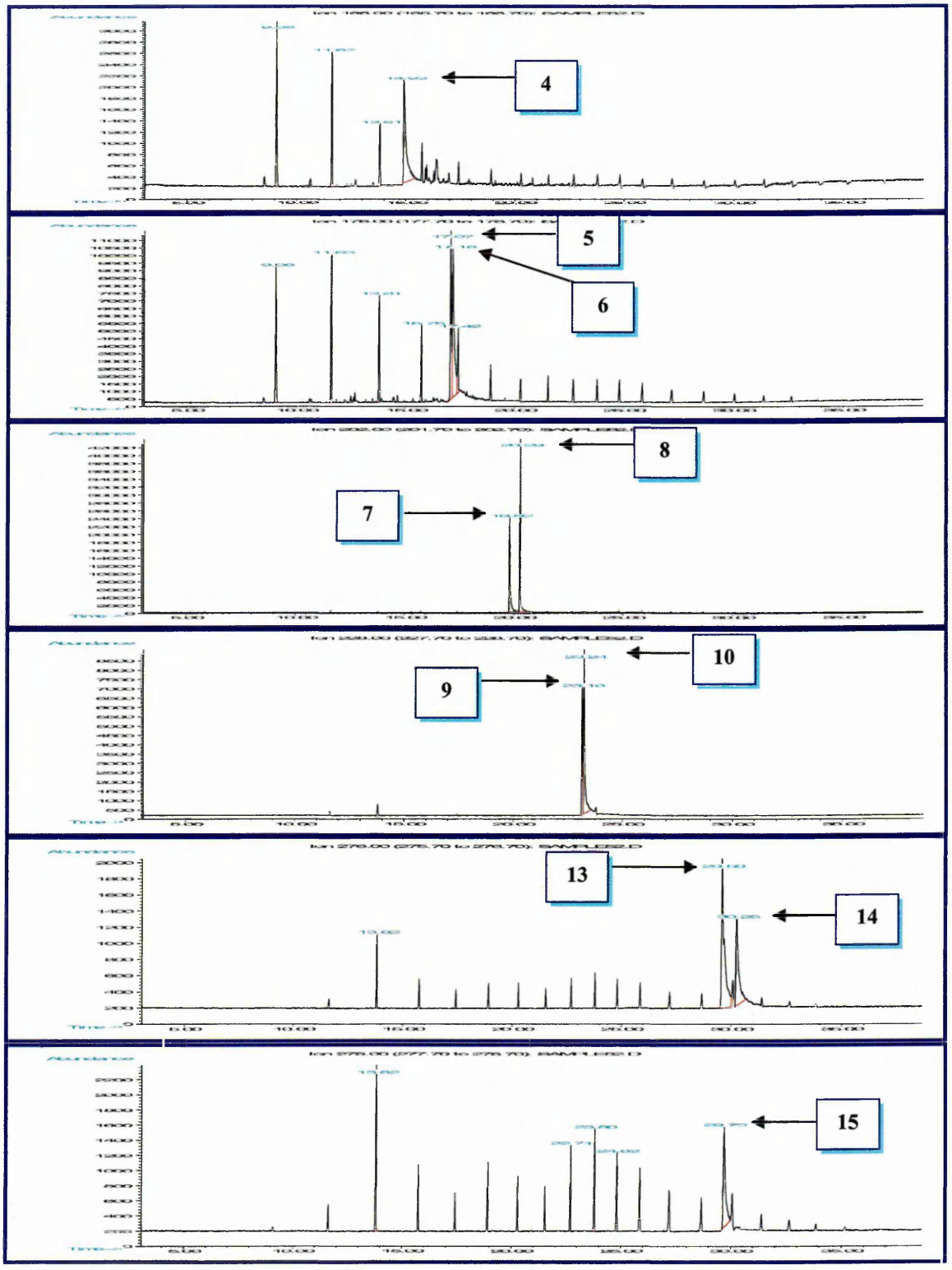
Appendix D
Ion chromatograms obtained by GC-MS for PAHs compounds extracted from produced water sample (SPT1). 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



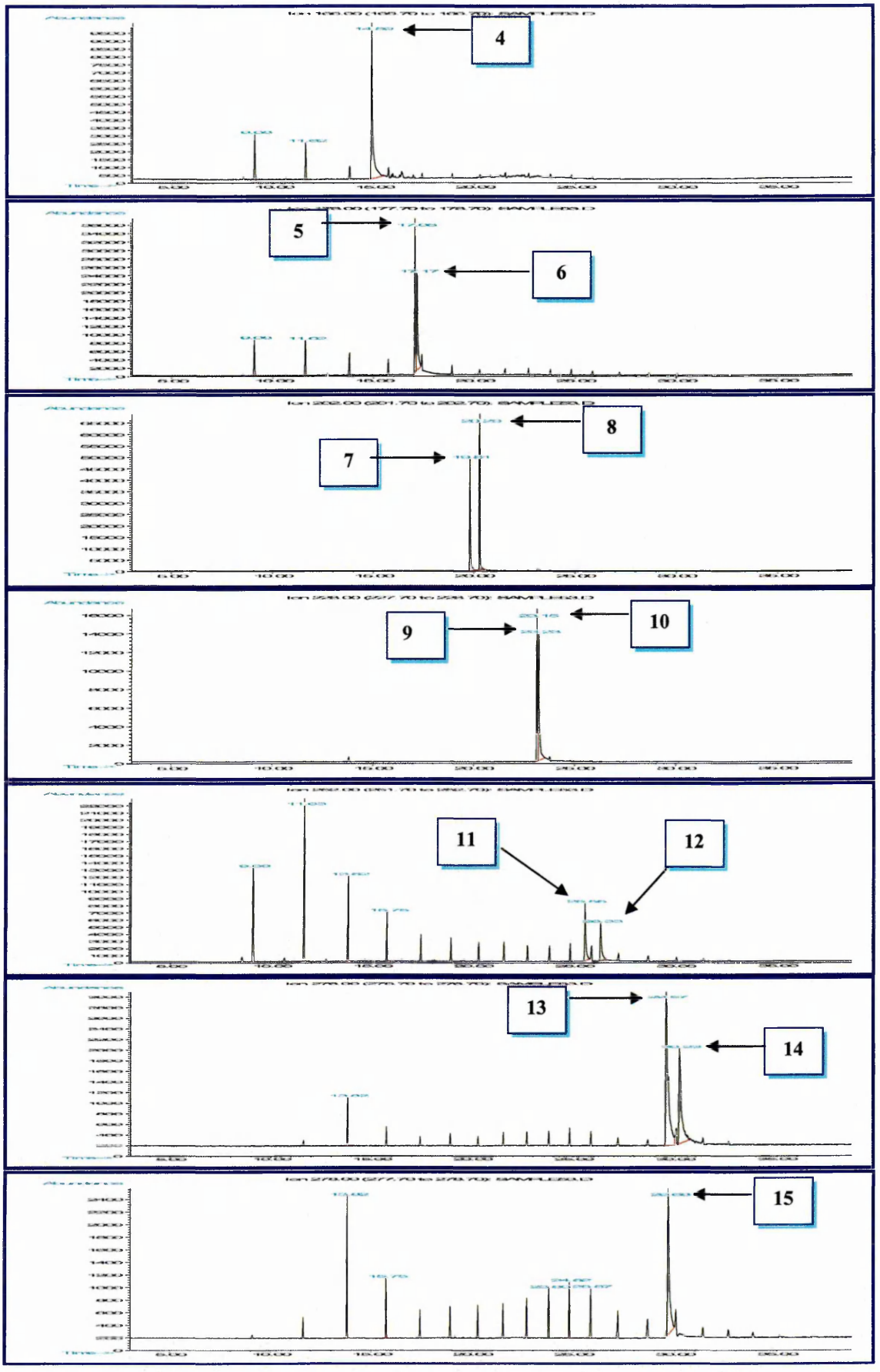
Appendix D1 Ion chromatograms obtained by GC-MS for PAHs compounds extracted from produced water sample (SPT1). 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10- CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP.



Appendix D2 Ion chromatograms obtained by GC-MS for PAHs compounds extracted from produced water sample (SPT2). 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 13-IP, 14- DBahA and 15- BghiP.



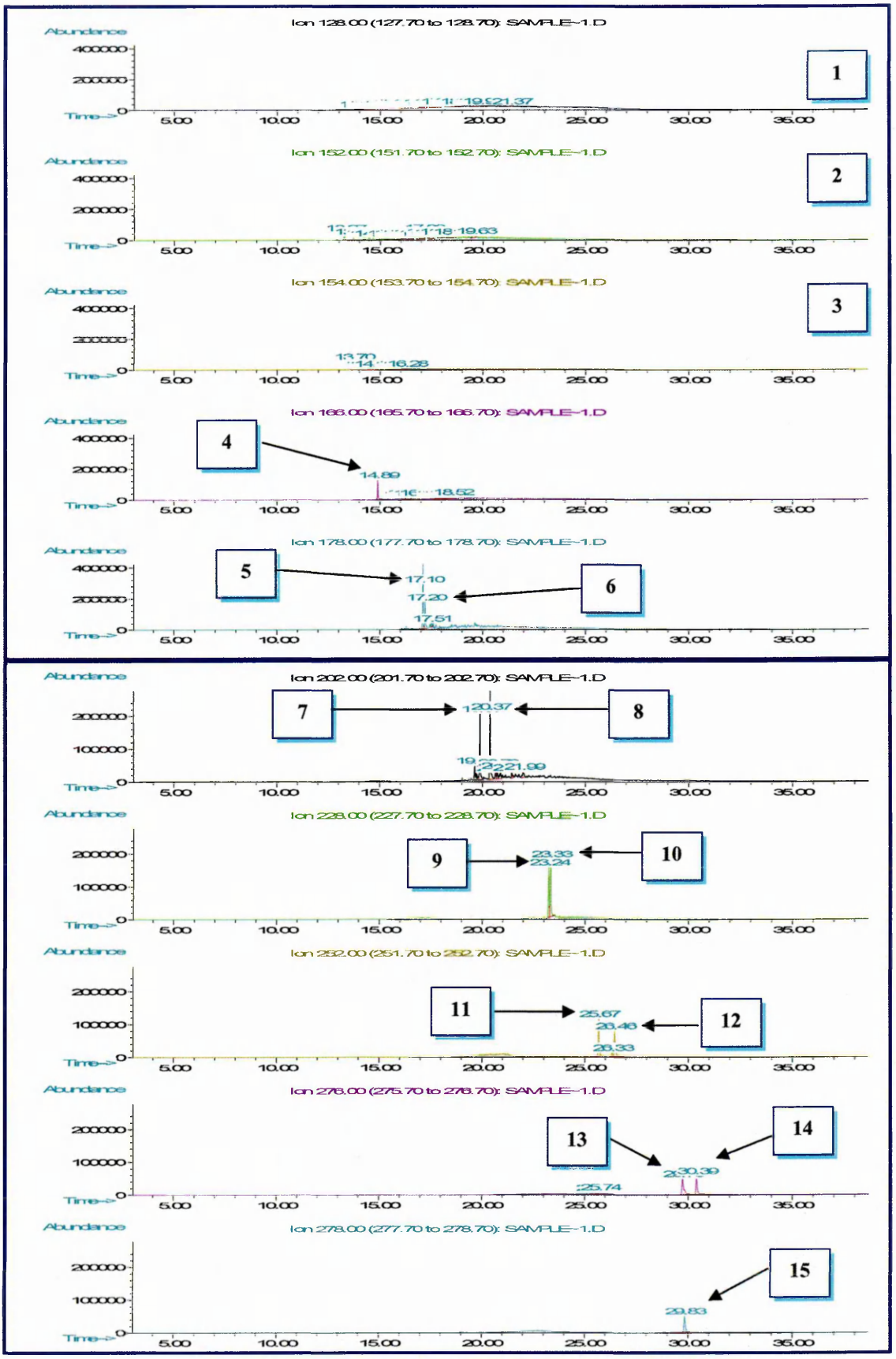
Appendix D3 Ion chromatograms obtained by GC-MS for PAHs compounds extracted from produced water sample (SPT3). 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 13- IP, 14- DBahA and 15- BghiP



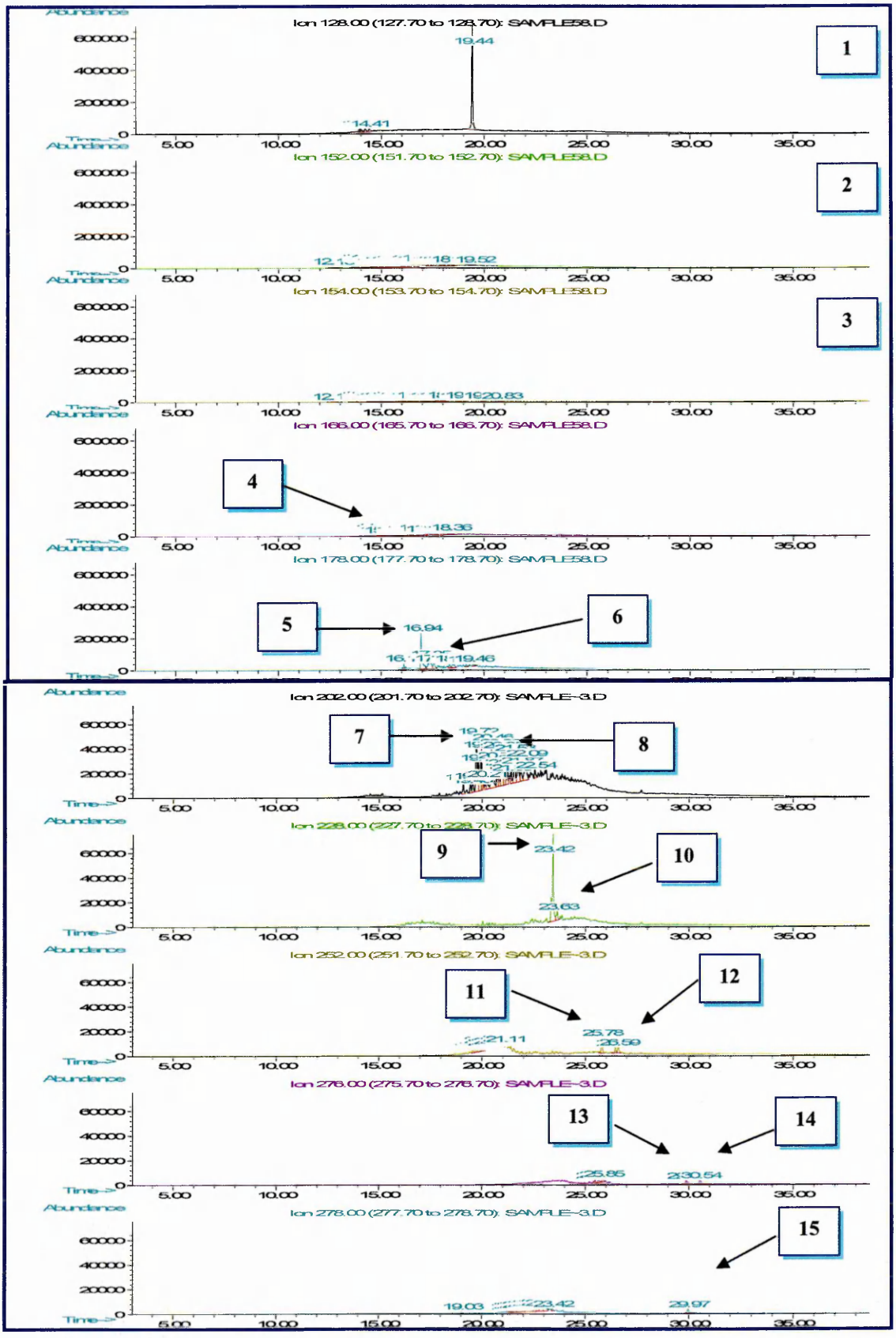
Appendix D4 Ion chromatograms obtained by GC-MS for PAHs compounds extracted from produced water sample (SPT4). 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP.

Appendix E

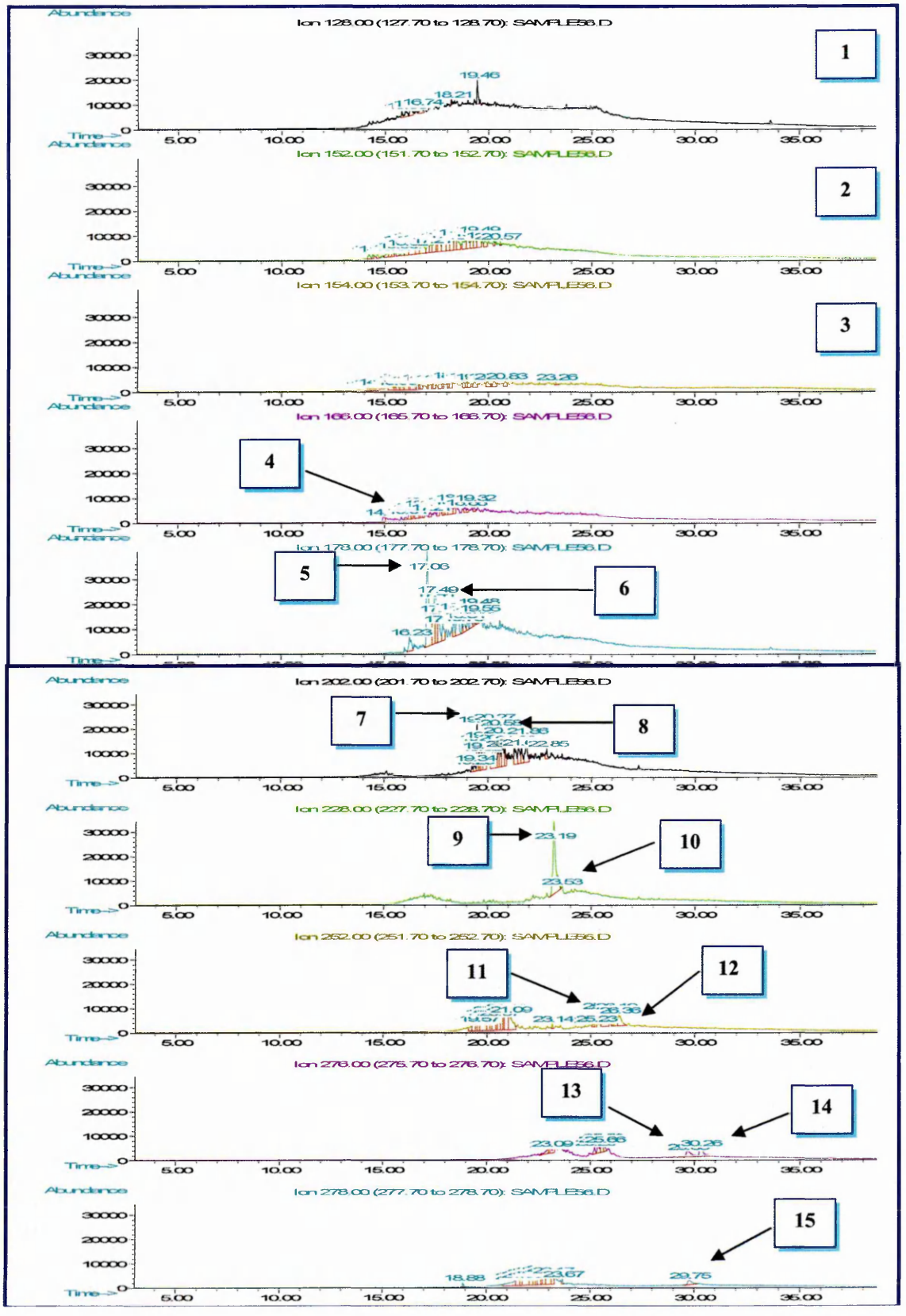
Ion chromatogram of extracted PAHs from soil sample (A2) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11- BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



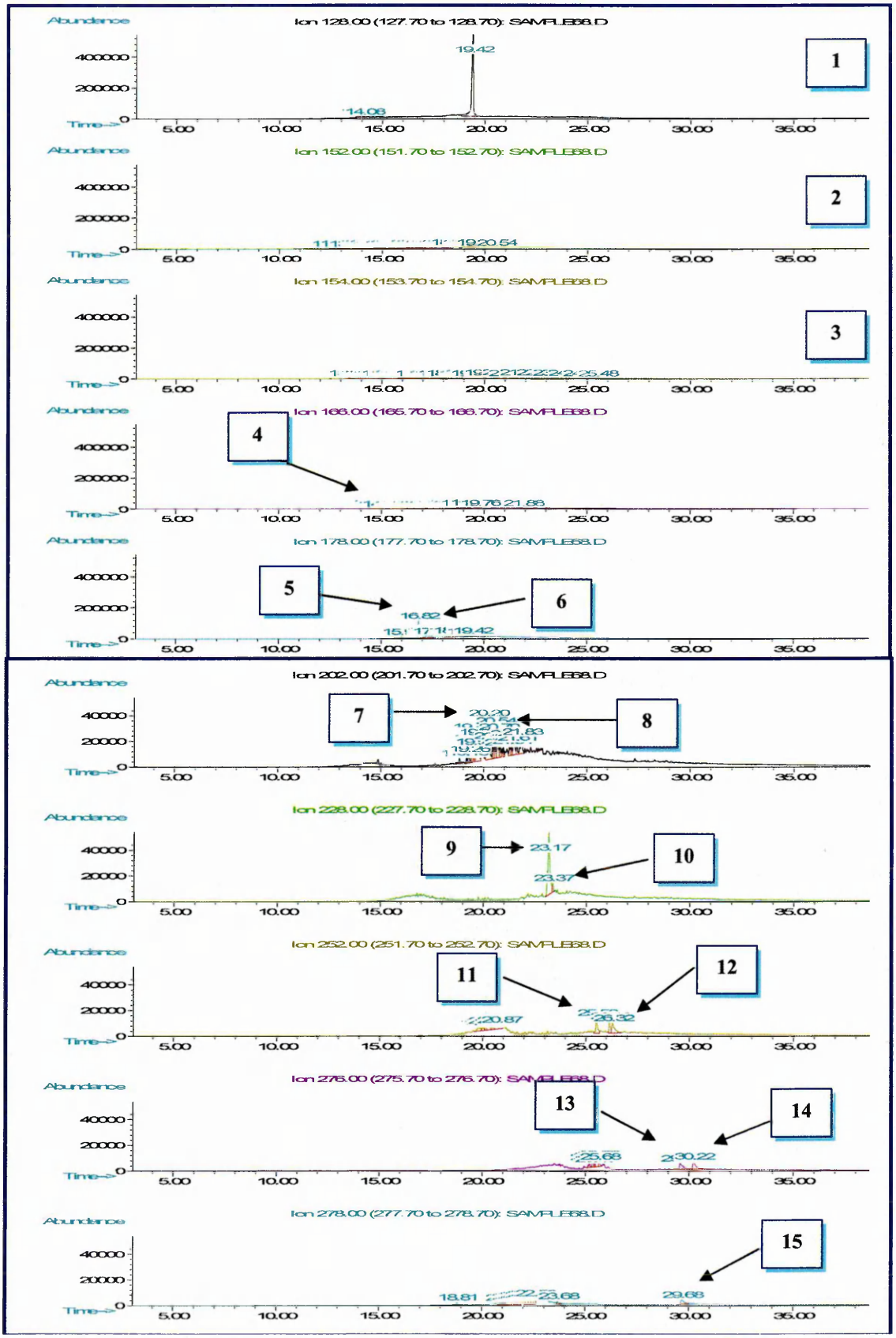
Appendix E1 Ion chromatogram of extracted PAHs from soil sample (A2) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



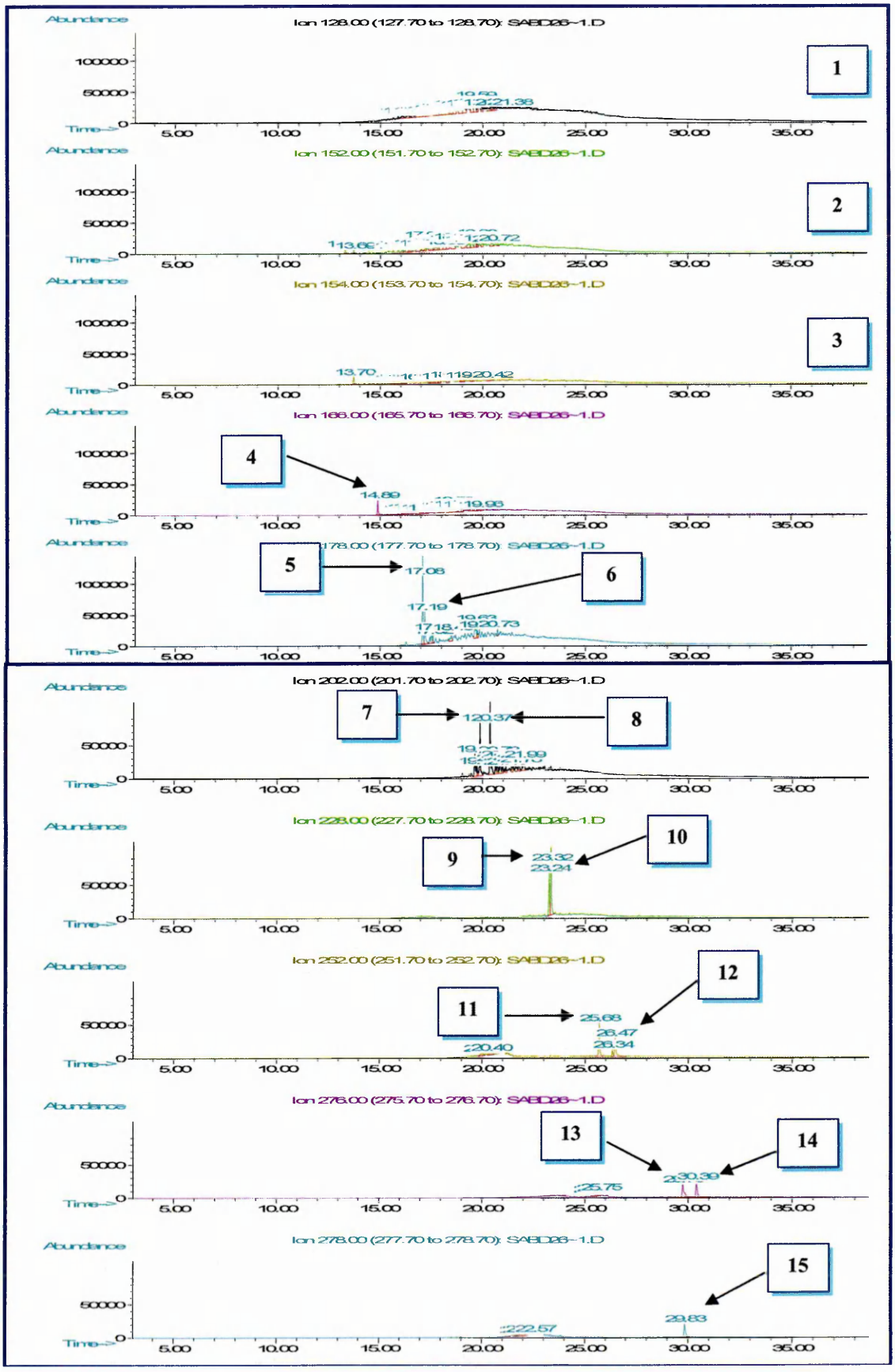
Appendix E2 Ion chromatogram of extracted PAHs from soil sample (A4) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



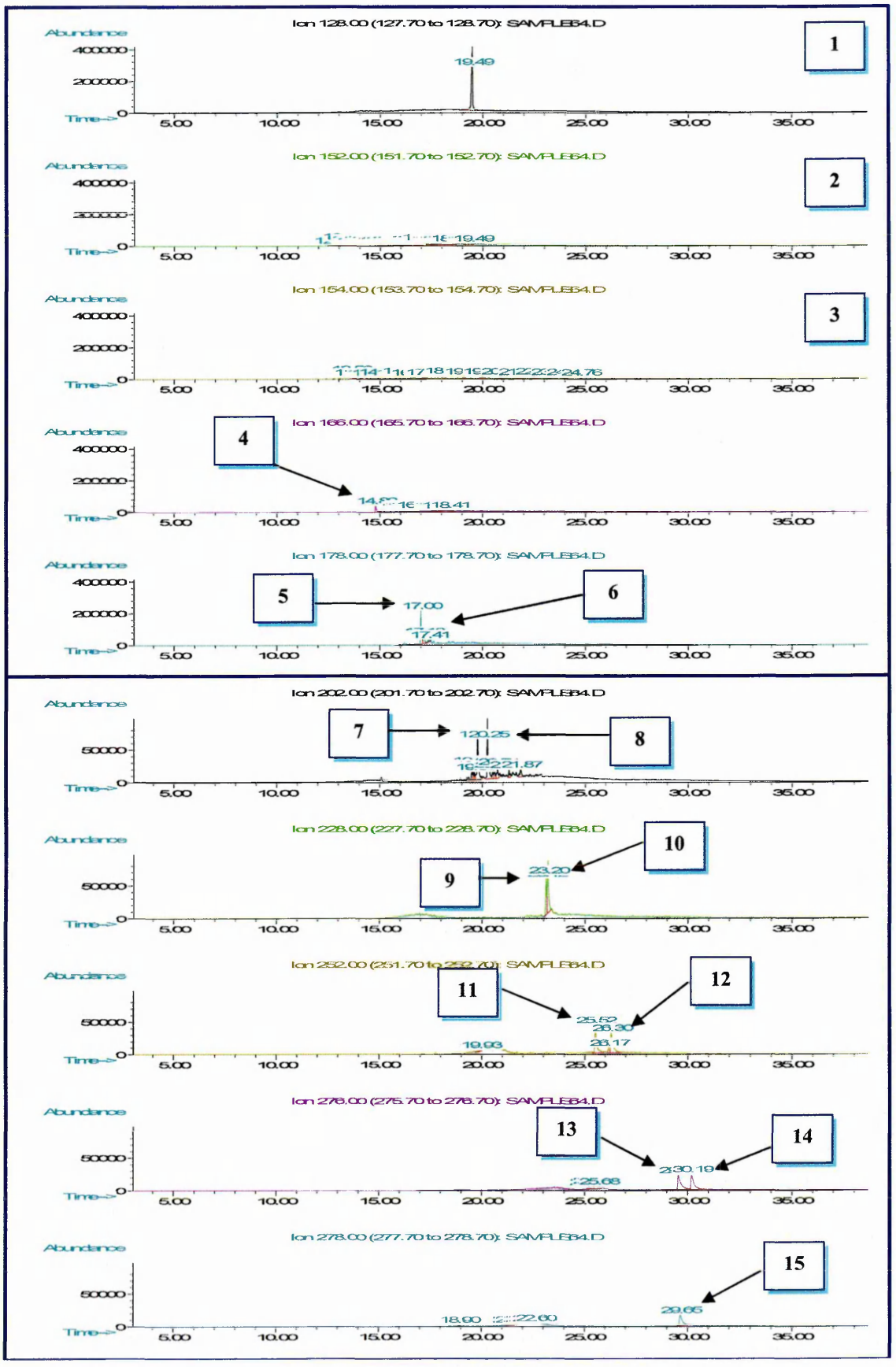
Appendix E3 Ion chromatogram of extracted PAHs from soil sample (A6) obtained byGC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



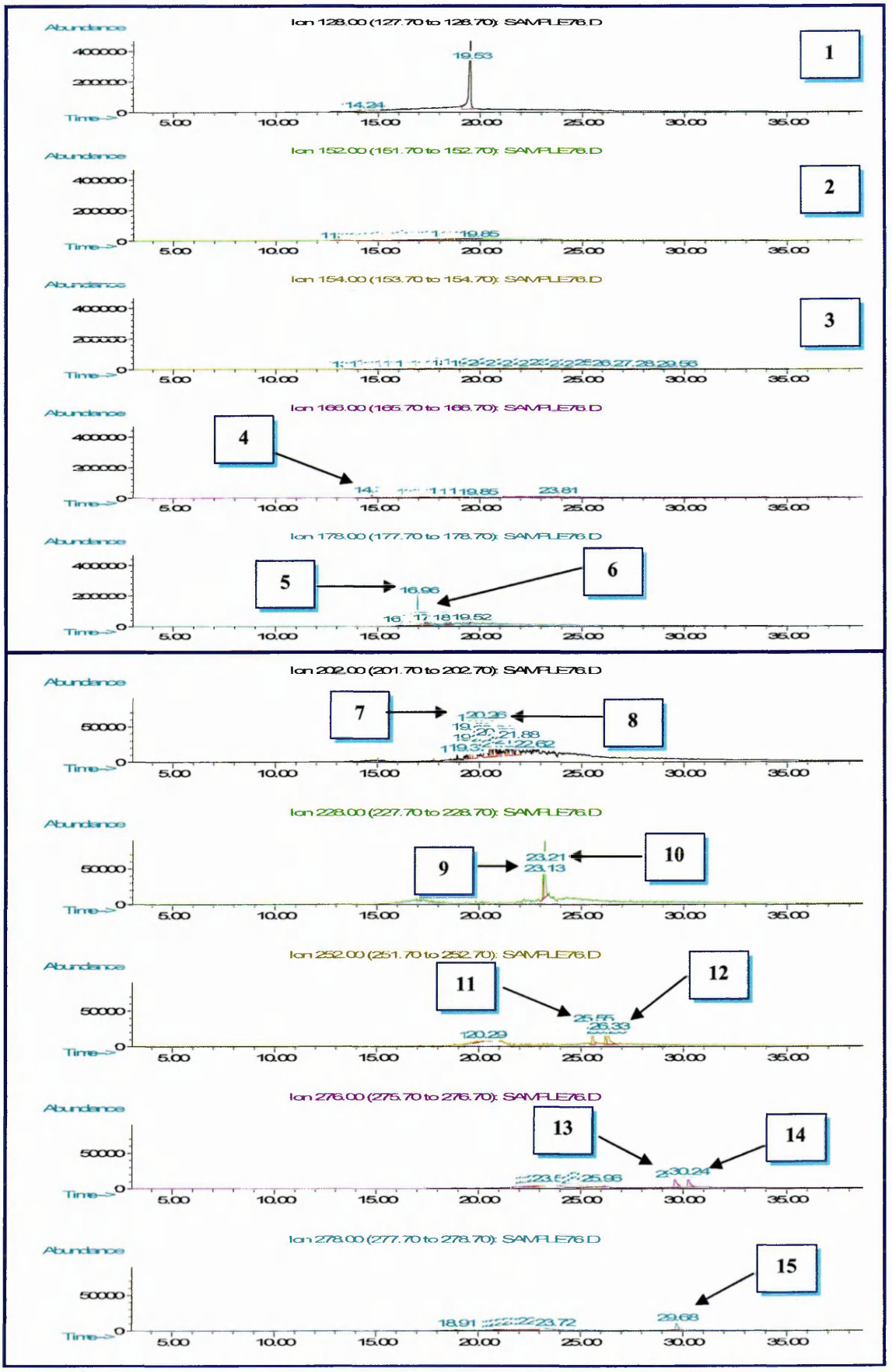
Appendix E4 Ion chromatogram of extracted PAHs from soil sample (B3) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



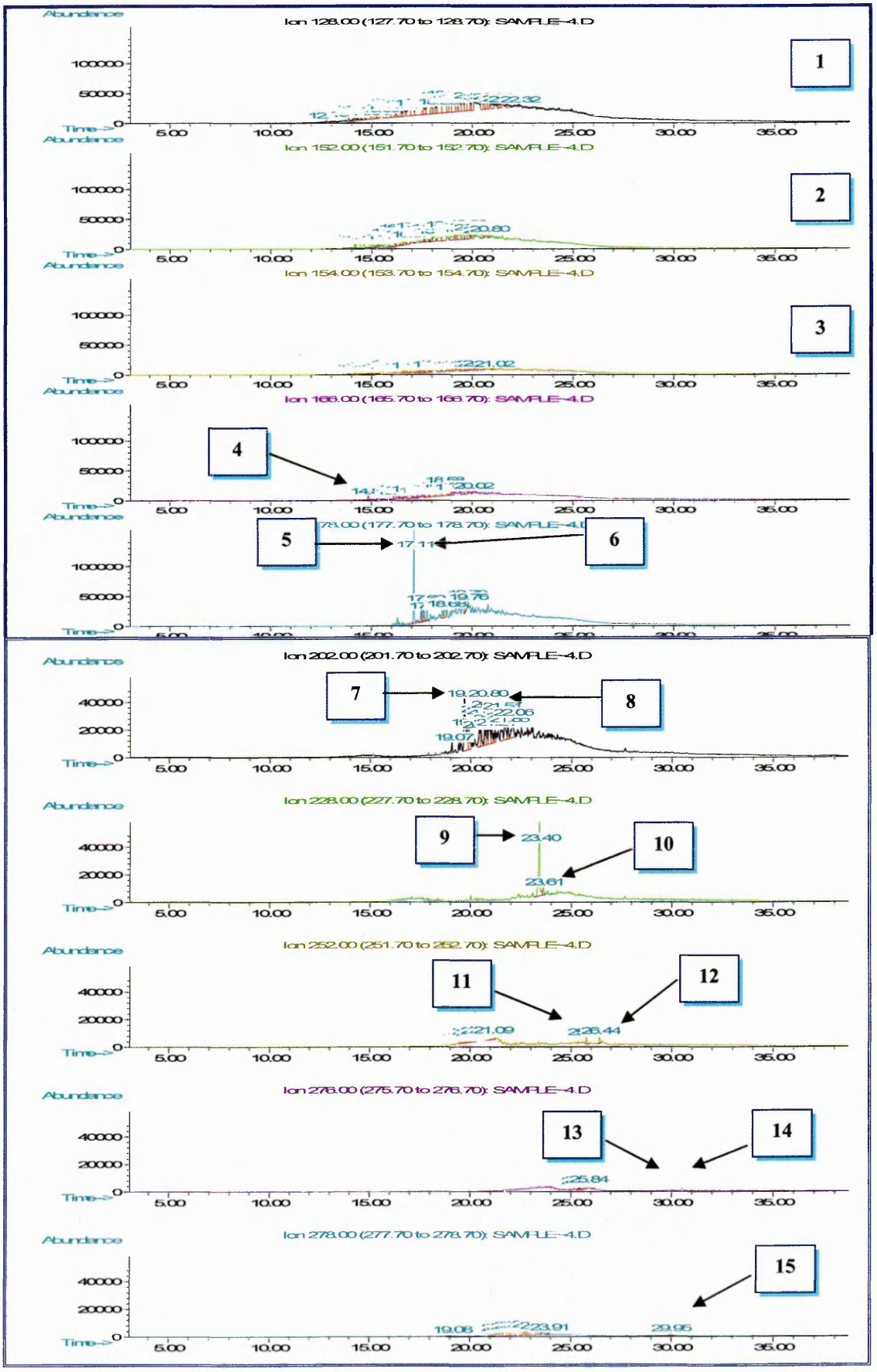
Appendix E5 Ion chromatogram of extracted PAHs from soil sample (B4) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



Appendix E6 Ion chromatogram of extracted PAHs from soil sample (C1) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



Appendix E7 Ion chromatogram of extracted PAHs from soil sample (C4) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP



Appendix E8 Ion chromatogram of extracted PAHs from soil sample (C6) obtained by GC-MS. 1- NAP, 2- ACL, 3- AC, 4- FL, 5- PHE, 6- AN, 7- FA, 8- PY, 9- BaA, 10-CHR, 11-BbFA, 12-BaP, 13- IP, 14- DBahA and 15- BghiP