Chapter I

Introduction

I. INTRODUCTION

I.1.History of Oil Spill

Marine pollution has been defined as: "the introduction by man, directly or indirectly, of substances or energy into the marine environment resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities, including fishing, impairment of quality for use of sea water and reduction of amenities. The first incident of pollution arising from damage to a ship appears to have occurred in the early eighteenth century, but the problem only came into prominence in the 1930's when more and more ships converted their boilers from coal to oil power. Since then there have been enormous increase in the scale of oil operations as a result of ever-increasing demands for energy. In the last decades, these changes have led to major pollution problems, "oil on the surface of the sea". The changes were gradual and the size of the pollution threat went almost unrecognized until a series of dramatic accidents brought the problem into the field of public concern. Among the major accidents

On 18 March 1967 - UNITED KINGDOM - The Torrey Canyon ran aground off Cornwall spilling 80,000 tonnes (919,000 barrels) of crude.

On 20 March 1970- SWEDEN - At least 438,000 barrels of oil spilled in a collision involving the Othello in Tralhavet Bay. Since that time and in spite of the international precautions several other accidents were recorded (Annex I).

The importance of studying water pollution by oil comes from the fact that marine life is greatly affected by oil spills. The biological responses that might accompany or follow an oil spill include **(Egaas and Varanis, 1982)**.

- Lethal toxic effects, where the components of the oil interfere with cellular or subcellular processes in the organisms to such an extent that death occurs.

- Sublethal effects that disturb the physiological or behavioural activities but do not cause immediate mortality.
- Uptake of the oil, or certain fractions of it by the organisms causing tainting or in some cases carcinogenesis.
- Transformation of pollutants to other members of the food web including man.
- Changes in biological habitats.

Lethal effects occure in the range of 1 to 10 mg/l. While sublethal effects will result at oil concentration as low as 0.1 to 1 mg/l (Thourya, 1986). Among the sublethal effects of petroleum hydrocarbons are:

- Delaying cellular divison in phytoplankton.
- Producing abnormal fish spawn.
- Reducing chemotactic feeding responses in snails and in crabs.
- Inhibiting mating responses of male crabs to sex pheromores and also decreasing filter feeding activity of muscles.

I.2.Oil pollution

Oil pollution is a severing global environmental problem causing a number of adverse negative impacts on human health, fisheries, aquacultures, tourism, ecosystem and eventually the national income. That is why water pollution with petroleum hydrocarbons have became the focus of increasing regulatory, public and research concern. Petroleum hydrocarbons are ubiquitous contaminants in the aquatic environment because of both human activities and natural processes (Moustafa[a], 2004). The aliphatic and polycyclic aromatic hydrocarbons (PAHs) fractions of dissolved petroleum are readily absorbed by most aquatic organisms because of their high lipid solubility and bio-concentration in fish and shellfish (Viguri et al., 2002). Aliphatic and PAHs have received a lot of attention due to their ubiquitous distribution in marine sediments (Wang et al., 2006).

The fate and behavior of spilled oils in the environment depend on a number of physicochemical and biological factors including evaporation,

dissolution, microbial degradation, photo-oxidation and interaction between oil and sediments. The combination of these processes, called weathering, alters the chemical composition of spilled oils. These changes have profound effects on the oil toxicity and biological impacts of the oil over the time and hence add great difficulties to the identification of the residual spilled oil in the impacted environment **(Wang et al., 1999)**.

I.3.Crude oil

Crude oil or petroleum (Black Gold) was formed under the surface of the earth millions of years ago. Constituents of crude oil were resulted from aerobic and anaerobic enzymatic degradation of organic matter under suitable conditions of temperature and pressure. Crude oils vary widely in appearance and viscosity from field to field. While all crude oils are essentially hydrocarbons, they differ in properties and in molecular structure **(Berger and Anderson, 1978)**. Crude oils are roughly classified into three groups, according to the nature of the hydrocarbons they contain. These are Paraffinic–Base Crude Oils, Asphaltic–Base Crude Oils, and Mixed–Base Crude Oils.

Crude oils are liquid but may contain gaseous or solid compounds or both in solution. The variation in properties of crude oils greatly affects the methods of refining, and the products obtained from it **(Van Der Have and Venner, 1975)**. Crude oil is a complex mixture of several thousand of chemical compounds **(Lewis and Aurnd, 1997)**. Hydrocarbons are the most abundant compounds, accounting for 50-98% of the total composition. While carbon (80-87%) and hydrogen (10-15%) are the main elements in petroleum, other components are found called non-hydrocarbons **(Clarck and Brown, 1977)**. Compounds containing N, S and O as constituents are often collectively referred to as NSO compounds. Crude oils also contain widely varying concentrations of trace metals such as V, Ni, Fe, Al, Na, Ca and Cu. Petroleum hydrocarbons consist of alkanes, cycloalkanes, and aromatic compounds containing at least one benzene ring Fig.(1).

I.3.1.The hydrocarbons

The alkanes, or aliphatic hydrocarbons Consist of fully saturated normal alkanes and branched alkanes of the general formula (CnH2n+2), with n ranging from 1 to usually around 40, although compounds with 60 carbons have been reported.

Many of the cycloalkanes or saturated ring structures, also called cycloparaffins or naphthenes are important constituents like that of the isoprenoids. They have specific animal or plant precursors (e.g., steranes, diterpanes and triterpanes) that serve as important molecular markers in oil spill and geochemical studies **(Albaiges and Albrecht, 1979)**.

I.3.1.1.Aromatic hydrocarbons

Aromatics are a class of hydrocarbons characterized by rings with six carbon atoms. Are usually less abundant than the saturated hydrocarbons, contain one or more aromatic (benzene) rings connected as fused rings (e.g., naphthalene) or lined rings (e.g., biphenyl). Aromatics are considered to be the most acutely toxic components of crude oil, and are also associated with chronic and carcinogenic effects. Many low-weight aromatics are soluble in water, increasing the potential for exposure to aquatic resources. Aromatics with two or more rings are referred to as polycyclic aromatic hydrocarbons. Petroleum contains many homologous series of aromatic hydrocarbons

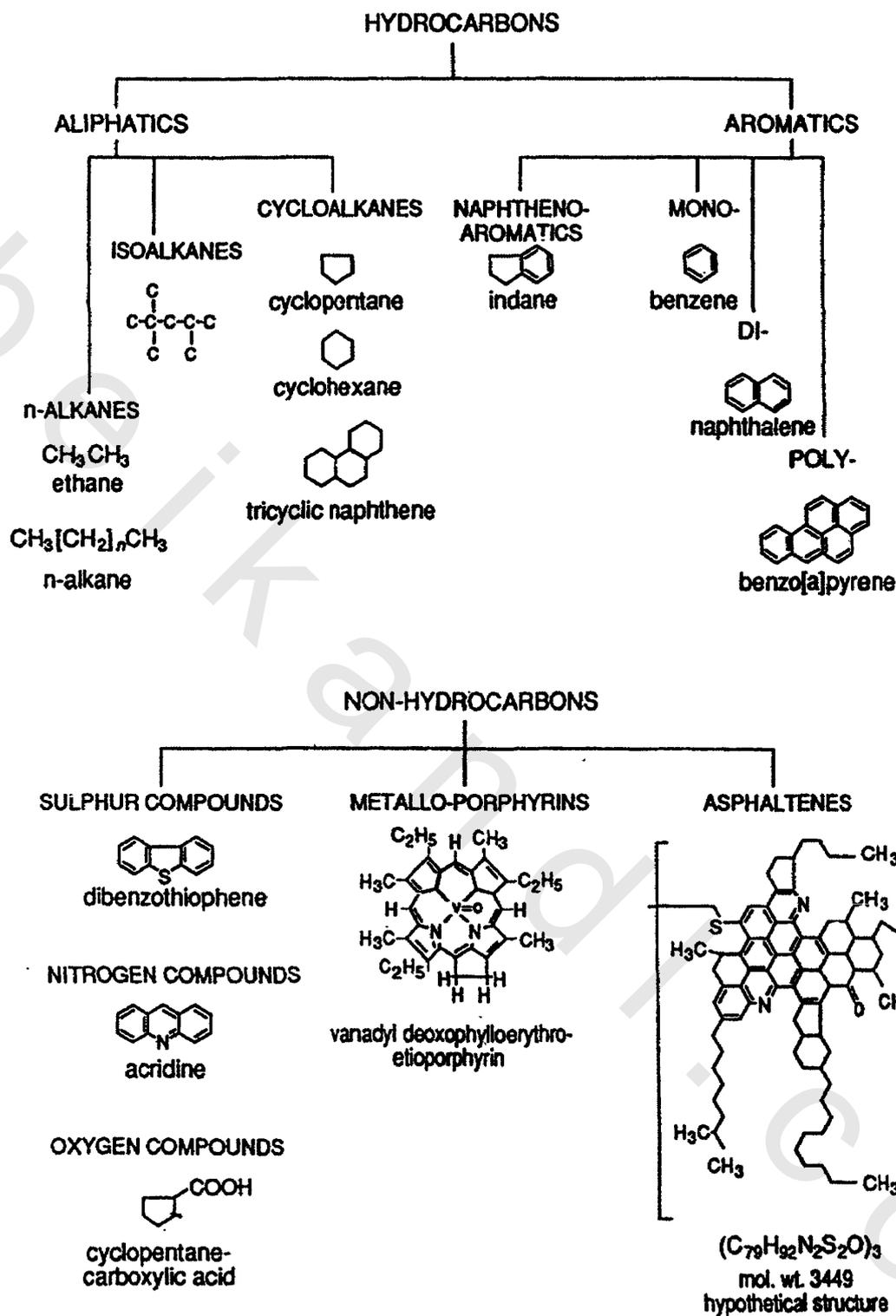I.3.1.1.1.Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a group of lipophilic anthropogenic compounds that are ubiquitously distributed in the environment. PAHs consist of two or more fused benzene rings in various arrangements. They form an important class of environmental contaminants, because some exhibit carcinogenic or mutagenic potential. There are several reports of increased incidence of cancer in marine animals from the vicinity of oil spills **(Colombo et al., 2005& Tfouni et al., 2007)**. Concern about PAHs in the environment arises also from the fact that many of them are
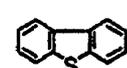
persistent. Because of their hydrophobic and persistent nature, they accumulate in bottom sediments of coastal and oceanic environments. Sedimentary PAHs are a source of exposure of PAHs directly to benthic organisms and indirectly (i.e. via resuspension) to pelagic organisms. The fact that many of them are persistent **(Boonyatumanond et al., 2006)**. Several PAHs may induce a number of adverse effects, such as immunotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity **(Qiao et al., 2006)**.
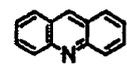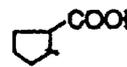
HYDROCARBONS

ALIPHATICS

AROMATICS

ISOALKANES

CYCLOALKANES

$$C-C-C-C-C$$

cyclopentane

NAPHTHENO-
AROMATICS

indane

MONO-

benzene

DI-

cyclohexane

n-ALKANES

CH_3CH_3
ethane

tricyclic naphthene

naphthalene

POLY-

$CH_3[CH_2]_nCH_3$
n-alkane

benzo[a]pyrene

NON-HYDROCARBONS

SULPHUR COMPOUNDS

dibenzothiophene

NITROGEN COMPOUNDS

acridine

OXYGEN COMPOUNDS

cyclopentane-
carboxylic acid

METALLO-PORPHYRINS

C_2H_5 H CH_3
H_3C C_2H_5
H N H
H_3C CH_3
C_2H_5 H H
H H
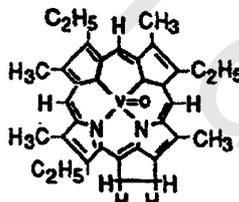
vanadyl deoxophylloerythro-
etioporphyrin

ASPHALTENES

CH_3
CH_3
S N
H_3C
N
CH_3
H_3C
CH_3
CH_3
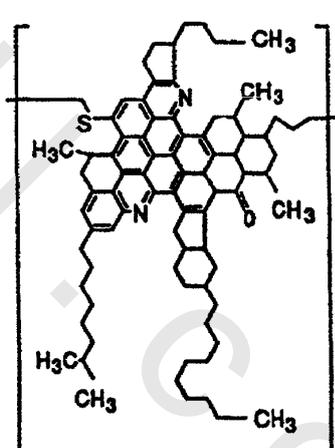
$(C_{79}H_{92}N_2S_2O)_3$
mol. wt. 3449
hypothetical structure

Fig.(1): Examples of the chemical structure of some common components of crude oil

Under this consideration, the United States Environmental Protection Agency (USEPA) has placed 16 of these PAHs in the priority-pollutant list. In the environment, PAHs may associate with particulates and dissolved organic matter and deposit onto sediments, the latter being a huge sink for air and waterborne contaminants that reflect the inputs for an ecosystem. In general, PAHs come mainly from two sources: petrogenic and pyrogenic. PAHs with four to six rings are derived generally from pyrogenic origin. The toxicity of PAHs to fish is of a particular interest since fish occupy an elemental position in relation to man and his food chain. Even minor concentrations of many PAHs could be accentuated through fish, posing a potential threat to man, being at the top of the atrophic hierarchy (**Samanta et al., 2002 & Maskaoui et al., 2002**).

I.3.1. The non- hydrocarbon

Are constituents of petroleum which can be grouped into six classes; sulfur compounds, nitrogen compounds, oxygen compounds, porphyrins, asphalthenes Fig.(1) and trace metals. They occur in crude oils and petroleum products in small quantities but some of them have considerable influence on product quality. Nitrogen is present in all crude oils in compounds as pyridines, quinolines, benzoquinolines, acridines, pyrroles, indoles, carbazoles, and benzocarbazoles (**Scholz et al., 1999**).

Sulfur compounds comprise the most important group of nonhydrocarbon constituents. In many cases they have harmful effects and must be removed or converted to less harmful compounds during refining process.

I.3.1.1. Asphaltenes

Are considered as part of the bottom of the barrel. They constitute the non-volatile, high molecular weight fraction of petroleum, from hundreds to thousands of daltons (Average molecular weight about 1000-15,000 daltons). Asphaltenes constitute the fraction of crude oil insoluble in n-alkane; they therefore remain dispersed in solid form in the crude (**Shirokoff et al., 1997**).

Asphaltene fraction is thought to be largely responsible for adverse properties of the oil; its high viscosity and its emulsion- polymer- and coke- forming propensity **(Jacobs and Filby, 1983 & Damste and De Leeuw, 1990& El-Gendy, 2004).**

Asphaltenes are dark brown to black, friable solid components of crude oils. Its specific gravity is close to one, and it is highly aromatic. Asphaltene molecules carry a core of stacked, flat sheets of condensed fused aromatic rings linked at their edges by chains of aliphatic and/or naphthenic-aromatic ring systems. The condensed sheets contain NSO atoms and probably vanadium and nickel complexes **(Strausz et al., 1999).** There are different models of asphaltene structure, that coincide in showing them as a system of condensed aromatic hydrocarbons with side chains up to C30, high proportion of heterocyclics such as carbazoles, thiophenes, cyclic terpenoids, sulfides, and porphyrins **(Speight, 1970& Ignasiak et al., 1977& Speight and Moschopedis, 1981& Pelet et al,. 1986& Cyr et al., 1987& Semple et al., 1990 & Murgich et al., 1999).** Also these compounds have a great influence upon the quality of crude oil, in such a way that they can lower its price and the price of its products if their concentration is too elevated, as Many of the problems associated with recovery, separation or processing of crude oil are related to the presence of high concentration of asphaltenes **(Flores and Howard, 2001).**

Environmental petroleum leakings are the most evident way by which asphaltenes and microorganisms get in touch. If we refer specifically to microorganisms, these compounds present an influence upon their distribution and activity, as they might either have a toxic effect upon the microorganisms or the microorganisms serve them as a source of carbon and energy.

One of the gravest problems related to these compounds in the environment, resides in their resistance to biodegradation by microbial metabolic activity. Due to this fact, metabolic routes involved in this process are the less known ones in these days, although, there is some evidence suggesting that some microorganisms have the potential capability of transforming asphaltenes, and in the best case, eliminating them **(Atlas, 1981).**

I.4. Fate and weathering of oil spill

Although the particular composition of a crude oil in its natural reservoir may be stable over geological time spans, its composition changes once exposed to water, oxygen, sunlight, microorganisms and other factors. The sum of all these physical, chemical and biological processes is known as weathering. Changes occur rapidly in the initial stages, but with diminishing speed as thermodynamic equilibrium with conditions in the environment is approached Fig.(2) schematically represents the fate of oil spilled. When crude oil or petroleum products are accidentally released to the environment, they are immediately subject to a wide variety of weathering processes **(Jordan and Payne 1980)**. These weathering processes can include (1) evaporation, (2) dissolution, (3) microbial degradation, (4) other processes such as dispersion and water-oil emulsification, photooxidation, adsorption onto suspended particulate materials and oil –mineral aggregation.

I.4.1. Spreading and advection

Spreading is the movement of the entire slick horizontally on the surface of the water due to effects of friction, viscosity, and surface tension. Advection is the movement of the oil due to the influence of overlying winds and/or underlying currents. Spreading dominates the initial stages of a spill and involves the whole oil, that is, it does not partition the various components of the oil or affect its chemical composition. Spreading and advection continue for approximately one week to ten days following the discharge, or until the oil is contained by shorelines, collection efforts, or other obstructions.

I.4.2. Evaporation

In the short term after a spill (hours to days), evaporation is the single most important and dominant weathering process, in particular for light petroleum products. In the first few days following a spill, the loss can be up to 40% and 70% of the volume of light crudes and petroleum products. For

heavy or residual oils the losses are only about 5-10% of its volume (**Fingas, 1995**).

I.4.3. Dissolution

The amount of the oil hydrocarbons dissolving in water phase from oil slick largely depends on the molecular structure and polarity of a given oil components, and the relative solubility of the oil component in water phase versus its solubility in the oil phase.
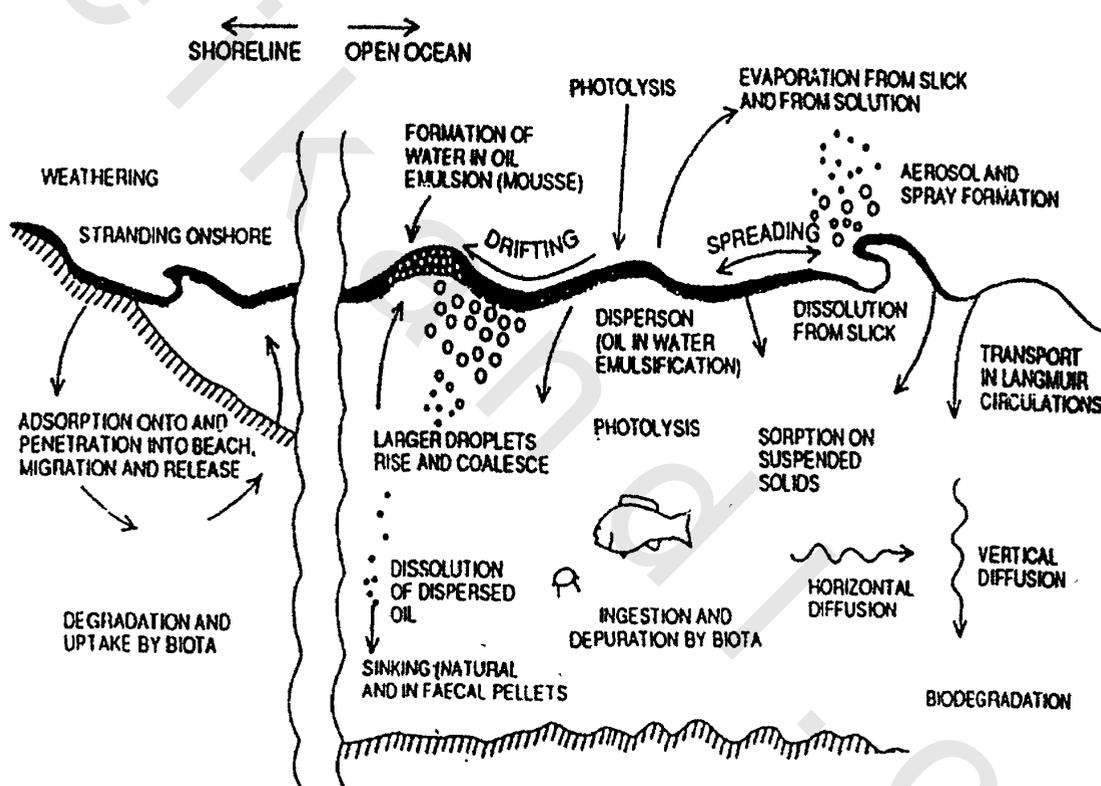

Fig.(2): Schematic diagram of oil spill processes at sea and shorelines

In general, the aromatic hydrocarbons are more soluble than aliphatic hydrocarbons and the solubilities increase as the alkylation of alkylated benzene or PAHs decrease. The lower molecular weight hydrocarbons are more soluble than the high molectilar weight hydrocarbons in that class. Therefore, it can be readily understood why Benzene, Toluene, Ethyl benzene, Xylene (BTEX) and lighter alkylbenzene compounds and some

smaller PAH compounds such as naphthalene are particulary susceptible to dissolution.

I.4.4.Natural dispersion

It is the process of forming small oil droplets that become incorporated into the water column in the form of dilute oil-in-water suspension. Dispersion reduces the volume of the slick at the surface but does not change the physiochemical properties of the oil. Following evaporation, it is the most important process in the breakup and disappearance of a slick. It begins soon after the spill occurs and reaches a maximum rate in approximately 10 hours following a spill.

I.4.5.Emulsification

Emulsification is the mixing of water droplets into oil spilled on the water surface. Such water-in-oil emulsions are highly viscous and have densities approaching seawater. Once the oil has emulsified, the weathering of oil can be significantly reduced. Emulsification begins during the first day of the spill and can continue to occur throughout the first year. The largest volume of emulsions is typically formed within the first week of the spill.

I.4.6.Photooxidation

It is considered to be another most important factor involved in the transformation of crude oil or its products released into the marine environment (Samanta et al., 2002). The photochemical degradation yields a great variety of oxidized compounds which are highly soluble in water.

I.4.7.Sedimentation and shoreline stranding

Sedimentation is the incorporation of oil within both suspended and bottom sediments. Sedimentation is a very important process in shallow, rough sea conditions where bottom sediments are repeatedly resuspended. It begins soon after the spill occurs and peaks several weeks after the spill.

Shoreline stranding is the visible accumulation of oil on shorelines following a spill. It is affected by the proximity of the spill to the shore, intensity of current and wave action on the affected shoreline, and the persistence of the spilled product.

I.4.8.Aggregation

Oil mineral aggregates (OMA) were found to result from interactions among the oil residues, fine mineral particle and seawater. OMA formation has now been identified as an important process that facilitates the natural removal of oil stranded in coastal sediments. OMA formation is enhanced by physical processes such as wave, energy, tides or currents. It has recently been noted that oil biodegradation may be enhanced by OMA formation.

All these processes alter the chemical composition of spilled oil, making the unambiguous identification of the source of oil something of challenge.

I.4.9.Biodegradation

Biodegradation of hydrocarbons by natural populations of microorganism represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants are eliminated from the environment **(Abalos et al., 2004)**. It is a complex process, whose quantitative and qualitative aspects depend on the type, nature and the amount of the oil or hydrocarbon present, the ambient and seasonal environmental conditions (such as temperature, oxygen, nutrients, water activity, salinity and pH), and the composition of the autochthonous microbial community. Hydrocarbons differ in their susceptibility to microbial attack. In general, the degradation of hydrocarbons is ranked in the following order of decreasing susceptibility: n-alkanes > branched alkanes > low-molecular weight aromatics > high- molecular weight aromatics and cyclic alkanes.

I.5.Physico-chemical properties of water

I.5.1. pH / Acidity / Alkalinity

pH is a measure of the amount of free hydrogen ions in water. Specially, pH is the negative logarithm of the molar concentration of hydrogen ions.Because pH is measured on a logarithmic scale, an increase of one unit indicates an increase of ten times the amount of hydrogen ions. A pH of 7 is considered to be neutral. Acidity increases as pH values decrease, and alkalinity increases as pH values increase. Most natural waters are buffered by a carbon-dioxide-bicarbonate system, since the carbon dioxide in the atmosphere serves as a source of carbonic acid.

$$H_2CO_3 \text{----> } HCO_3^- + H^+ \qquad pK\sim7.5$$
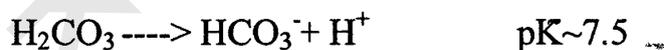
This reaction tends to keep pH of most waters around 7-7.5, unless large amounts of acid or base are added to the water. Most streams draining coniferous woodlands tend to be slightly acidic (6.8 to 6.5) due to organic acids produced by the decaying of organic matter. In waters with high algal concentration, pH varies diurnally; reaching values as high as 10 during the day when algae are using carbon dioxide in photosynthesis and drops during the night when the algae respire and produce carbon dioxide.

The pH of water affects the solubility of many toxic and nutritive chemicals; therefore, the availability of these substances to aquatic organisms is affected. As acidity increases most metals become more water soluble and more toxic. Toxicity of cyanides and sulfides also increases with a decrease in pH (increase in acidity). Ammonia, however, becomes more toxic with only a slight increase in pH.

Alkalinity is the capacity to neutralize acids, and the alkalinity of natural water is derived principally from the salts of weak acids. Hydroxides carbonates, and bicarbonates are the dominant source of natural alkalinity. Reactions of carbon dioxide with calcium or magnesium carbonate in the soil create considerable amounts of bicarbonates in the soil. Organic acids such as

humic acid, also form salts that increase alkalinity. Alkalinity itself has little public health significance, although highly alkaline waters are unpalatable and can cause gastrointestinal discomfort.

I.5.2. Conductance or Electrical Conductivity (EC) and Dissolved Solids

Conductivity is the ability of a substance to conduct electricity. The conductivity of water is a more-or-less linear function of the concentration of dissolved ions. Conductivity itself is not a human or aquatic health concern, but because it is easily measured, it can serve as an indicator of other water quality problems. If the conductivity of a stream suddenly increases, it indicates that there is a source of dissolved ions in the vicinity. Therefore, conductivity measurements can be used as a quick way to locate potential water quality problems. Conductivity is measured in terms of conductivity per unit length, and meters typically use the units microsiemens/cm. All natural waters contain some dissolved solids due to the dissolution and weathering of rock and soil. Dissolved solids are determined by evaporating a known volume of water and weighing the residue. Some but not the entire dissolved solids acts as conductors and contribute to conductance. Waters with high total dissolved solids (TDS) are unpalatable and potentially unhealthy. Water treatment plants use flocculants to aggregate suspended and dissolved solids into particles large enough to settle out of the water column in settling tanks. A flocculent is a chemical that uses double-layer kinetics to attract charged particles.

I.5.3.Temperature

Metabolic rate and the reproductive activities of aquatic life are controlled by water temperature. Metabolic activity increases with a rise in temperature, thus increasing a fish's demand for oxygen; however; an increase in stream temperature also causes a decrease in (dissolved oxygen) DO, limiting the amount of oxygen available to these aquatic organisms with a limited amount of DO available, the fish in this system will become stressed. A rise in temperature can also provide conditions for the growth of disease-causing organisms.

Water temperature varies with season, elevation, geographic location, and climatic conditions and is influenced by stream flow, streamside vegetation, groundwater inputs, and water effluent from industrial activities. Water temperatures rise when streamside vegetation is removed.

I.6. The origin and significance of ionic constituents in water

I.6.1. Total dissolved salts (TDS)

Salinity is an important property of industrial and natural waters. It was originally conceived as a measure of the mass of dissolved salts in a given mass of solution. The experimental determination of the salt content by drying and weighting presents some difficulties due to the loss of some components. The only reliable way to determine the true or absolute salinity of natural water is to make a complete chemical analysis (**Metwaly, 2004**).

Water salinity is the sum of all mineral substances detected by the chemical analysis, total solids (TS) and total dissolved solids (TDS) are equivalent terms. Different methods are used for water classification according to its salinity values. According to **Chebotarev, 1955**, the natural water is classified into three main categories of total salinity; fresh water (TDS up to 1500 mg/1), brackish water (TDS from 1500 to 5000 mg]) and saline water (TDS more than 5000 mg/1).

I.6.2. Sodium

Sodium is the most abundant member of the alkali- metal group of the periodic table. It doesn't exist in native state but is present only as compounds in rocks and deposits such as halite (NaCI), thenardite (Na_2SO_4) and albite ($NaSi_3O_8$). Also, industrial wastes represent one of the main sources of sodium in water. The high solubility of sodium salts and the limited degree of sorptive bonding of sodium onto clay minerals and other adsorbents lead to a considering enrichment in the sea. the sodium concentrations in natural water can have a very wide range from less than 1

mg/l in rain water and dilute stream runoff in areas of high rainfall to very high levels in brines associated with evaporated deposits and in brines of closed basins, where more than 100,000 mg/l may be present.

I.6.3. Potassium

In most natural water, the concentration of potassium is much lower than the concentration of sodium. In opposite to sodium, potassium ions tend to become fixed, through sorption, on clay minerals. This is reflected in its relative abundance in clays just as in its relative scarcity in sea water.

I.6.4. Calcium

Calcium is the fifth most abundant element in the earth crust and soils, and it is readily leached out by rain water, so it is present in all surface waters at varying concentrations. In surface water, calcium is one of the most abundant cations because of the weathering of rocks and soils. The concentration of Ca^{2+} in natural water is usually not greater than 1000 mg/l **(Hem, 1989)**.

I.6.5. Magnesium

Magnesium is the eighth most abundant elements in the earth. It is mainly presented as dolomite $(CaMg(CO_3)_2)$ and magnesite $(MgCO_3)$. Magnesium is present in surface waters and underground waters. The concentration of magnesium in natural water is generally less than 50mg/l, ocean water contains more than 1000 mg/l, and brines may contains as much as 75,000 mg/l magnesium **(Hem, 1989)**.
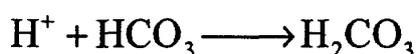
I.6.5. Bicarbonate and carbonate

Carbonates and bicarbonates are common to most waters due to the abundance of carbonate minerals in nature (limestone and dolomite) as well as carbon dioxide.

The system $CO_2 + H_2O = H_2CO_3 = HCO_3^- + H^+ = CO_3^{2-} + H^+$ is very important in water, controlling the various species that originates from carbonate in rocks and free carbon dioxide. The total CO_2 content in water is usually subdivided into free and bound CO_2. Free CO_2 comprises dissolved CO_2 and undissociated H_2CO_3, while bound CO_2 includes the hydrogen carbonate (bicarbonates) and carbonate ions. Free CO_2 in groundwater has various origins. It may originate from atmospheric CO_2 that becomes dissolved in rainwater or from ground air dissolving in the percolating water, or directly from groundwater. Free CO_2 is produced by anaerobic or aerobic oxidation of organic substances, fossilized organic deposits by man-made organic pollution and by the effect of organic acids such as humic acids and naphthalinic acids **(Smith et al, 1930& Metwaly, 2004)** or inorganic acids (sulfuric and nitric acids) on carbonates.The bicarbonate ion is the principal alkaline constituent of almost all water supplies. It is generally found in the range of 5 to 500 mg/l, as $CaCO_3$. The dissociation of bicarbonate to carbonate ions becomes predominant above pH of 8.2; below this the ratio of bicarbonate to carbonate ions increases to more than 100 to 1 at pH of 6 and below. The part of alkalinity titration above pH of 8.2 is therefore, a measure of carbonate ions present and that below 8.2 is largely a measure of bicarbonate ions. Below pH 4.5 most of the bicarbonate ions are converted to carbonic acid molecules **(Davis and De Wiest, 1966)**.

$$H^+ + HCO_3 \longrightarrow H_2CO_3$$

The concentration of bicarbonate for natural water generally is held within a moderate range by the effect of carbonate equilibria.

I.6.6. Chloride

The chloride ion is widely distributed in the environment, mainly as its salt with sodium, potassium and calcium. The element chlorine is the most abundant of the halogens. Chloride ion is present in all natural waters with an average concentration of 3 mg/l in rainwater and 19000-mg/l in seawater **(Correns, 1956&Metwaly, 2004)**.

A significant fact illustrating the geochemical behavior of this element is that more than three-fourths of the total amount present in the earth's outer crust, atmosphere and hydrosphere is in solution in the ocean as Cl^- ions, which forms ion pairs or complex ions with some of the Cations present in natural water.

I.6.7. Sulfate

Sulfate ions are widely distributed in surface waters, where they normally occur at concentration levels of 10-180 mg/l. They are introduced into water from leaching of rocks and soils, from atmospheric precipitates, from sewage and industrial discharges.

I.7. Techniques used for the characterization of polluted samples

Despite the rapid advances in recent years in analytical technology and in our understanding of the environmental fields, oil spill identification still needs more efforts **(Wang and Fingas, 2003)**. Because crude petroleum and its products represent an extremely complex and variable mixture of chemical compounds, mostly organic, no one single technique that will quantify all the components (especially hydrocarbons) polluting aquatic environment can be used sufficiently **(Kathryn, 1993)**. The choice of specific techniques from among the multiplicity of analytical procedures depends on many factors such as sampling methods, personal analytical performance, the aim of analysis and perhaps most important, the availability of instrumentation.

The first step in assessing the seriousness of petroleum hydrocarbons pollution in the aquatic environment involves determining the magnitude of its concentrations.

Chemical fingerprinting is the application of analytical chemistry to identify the sources of complex environmental pollutants, including petroleum. These techniques have been in use for the mentioned decades **(Boehm et al. 1997)**. Chemical fingerprinting of petroleum has now evolved into a science where the original source(s) of petroleum can often be

identified by the relative abundance of key individual compounds(e.g. hydrocarbons), trace metals (vanadium over nickel ratio) or markers in the materials being analyzed.

I.7.1.Gravimetric Techniques

Gravimetric methods measure extractable matter by a solvent, not removed during solvent evaporation and capable of weighed. The advantage of gravimetric methods is that they are simple, quick, and inexpensive. These methods are not especially suitable for measurement of light hydrocarbons that volatilize at temperatures below 70-85°C. Gravimetric methods give no information on the type of fuel present, no information about the presence or absence of toxic compounds, and no specific information about potential risk associated with the contamination. Gravimetric oil and grease methods measure anything that dissolve in the solvent and remains after solvent evaporation. These substances include hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related biogenic materials. Because extracts are heated to remove solvent, these methods are not suitable for measurement of light hydrocarbons (i.e. less than C15) that volatilize at temperatures below 70-85°C.

I.7.2.Thin layer chromatography (TLC) fingerprinting

TLC holds a prominent place technique for the isolation and detection of petroleum hydrocarbons in a wide variety of environmental matrices **(Berrut and Jonathan, 1984)**. It is applicable to all compounds regardless of volatility. Another advantage of TLC is the lack of a need for expensive equipment and it is more efficient and less time consuming than alternative chromatographic techniques due to the minimal clean up required prior analysis. In general, it can be stated that there is no universal method which is superior to another. The choice of a particular system or systems will depend upon the hydrocarbon to be separated and the chemical characteristics of the background material from which they are to be isolated. Preparative TLC was used to obtain saturated hydrocarbons, aromatic hydrocarbons and polar compounds from a pollutant sample **(Whittaker and Pollard, 1994&**

Bharrut et al., 1997). Quantitative hydrocarbon group types analysis of petroleum hydrocarbons was performed by TLC- FID **(Mansfield et al., 1999)**.

I.7.3. Gas chromatography (GC)

The most widely used methods for hydrocarbon detection are based on gas chromatography **(Bacaud and Rouleau, 1996& Nouwen et al., 2004)**. It has a number of advantages over other separation techniques. It is fast and extremely sensitive due to the variety of detectors, which are still generally unavailable to other forms of chromatography. Both packed and capillary columns have been used in an effort to attain high resolution of the n-alkane fingerprinting components. The advantages in capillary columns over packed columns are in obtaining practically improved separations in order to give fine structured chromatographic fingerprints useful for source recognition. GC enables one to determine specific characteristic patterns for the contamination and the source **(Trabelsi and Driss, 2005)**. The chemical nature of hydrocarbon pollutants is influenced by the type of crude oil spilled and the environmental conditions affecting the nature and degree of weathering. Real spill samples may have their fingerprint patterns distorted by the so-called weathering effects. It can drastically alter the profiles of the chromatogram obtained.

The flame ionization detector (FID) is a nonselective detector that employs a hydrogen-fueled flame to ionize organic compounds. The advantage of GC/FID is its sensitivity to a broad range of hydrocarbon compounds. GC/FID systems are sometimes used to measure PAHs, with a typical detection limit of 10μg/L in water and 330μg/kg in soil. Flame ionization detectors are destructive detection systems because the analyte is combusted during detection. They may only be used as the second detector in cases where a GC is equipped with two detectors in series.

Gas chromatographic profile is of prime importance to detect the origin of pollutant hydrocarbons (petrogenic or biogenic). It is possible to identify petrogenic contamination by looking at n-alkane profiles **(Russ, 1985)**. The

gas chromatograms of petrogenic origin are characterized by broad spectrum of regularly spaced aliphatic hydrocarbon peaks, protruding over an unresolved envelop (hump) known as unresolved complex mixture (UCM). The hump comprising the complex mixture of unseparated naphthenic and aromatic hydrocarbons **(George et al., 1990)**. However, biogenic n-alkanes are dominated by odd numbered carbon atoms, shorter chain nC_{25} reflecting a phytoplankton input and a larger chain nC_{25} a terrestrial or vascular plant input. The absence of homologus series of n-alkanes may indicate that the sample is either very highly weathered crude oil or is not contaminated by crude petroleum. Also, the two isoprenoids (pristane and phytane) may serve as molecular markers for either biogenic or petroleum pollutants. Since pristane is the dominant isoprenoid in zooplankton, phytane acts as a marker to recognize contamination by petroleum. Terrestrial plant waxes, aquatic phytoplankton, biomass combustion and natural oil seeps contribute to natural inputs of hydrocarbons including aliphatic and aromatic hydrocarbons **(Masters and Root, 1987& Waldo et al., 1991)**.

The gas chromatographic profile can also be used to detect the type of pollutants and give an idea about the degree of weathering as follows:

1) Waxy crude oil shows high n-paraffin peaks with relatively small envelope (UCM), chromatogram of Fig.(3).

2) Weathered crude oil patterns, show comparatively less abundant n-paraffin peaks, chromatogram of Fig.(3).

3) Very highly weathered crude oil pattern, show minor or reduced peaks with an increase of the UCM hump, chromatogram of Fig.(3).

4) Crude oil residues from tank washings show additional maximum of well-resolved n-alkanes in the higher boiling region. These paraffins originate from crystallization on cold walls in tankers and are washed out with the residues from the original crude oil, chromatogram of Fig.(4).

5) Used lubricating oil can be detected by an unresolved hump in the higher boiling region with small peaks of n-paraffins, chromatogram of Fig.(5).

6) Light petroleum fractions, show n-paraffin peaks in the range of low molecular weight compounds Fig.(6), light fuel oil is easily detected in

GC screening because of its limited boiling range: compounds end at about nC_{25}.

7) Chromatogram of biogenic pollutant Fig.(7).
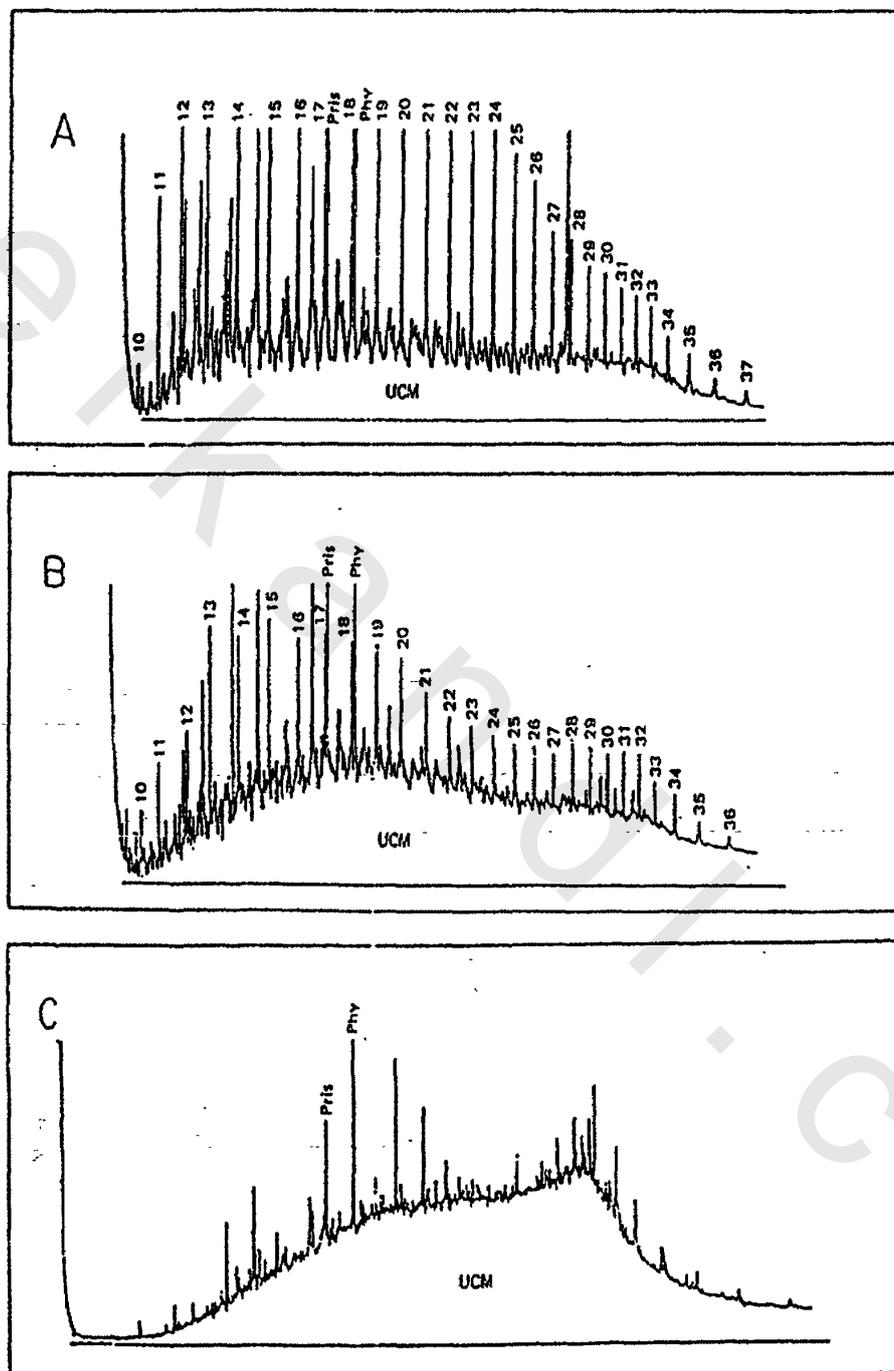
Fig.(3): Gas chromatograms for (A) waxy crude oil, (B) weathered and (C) very highly weathered crude oil.

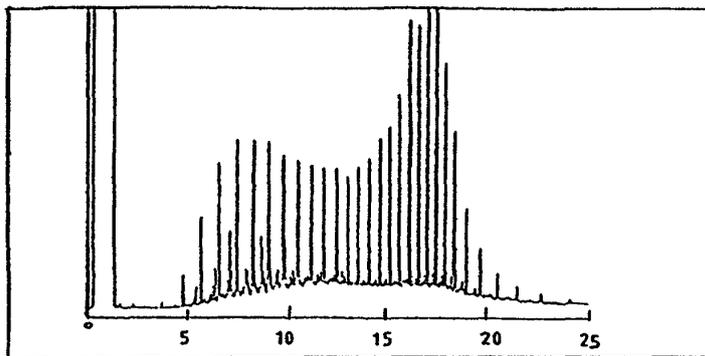**Fig.(4):**Gas chromatogram for tank washing water.
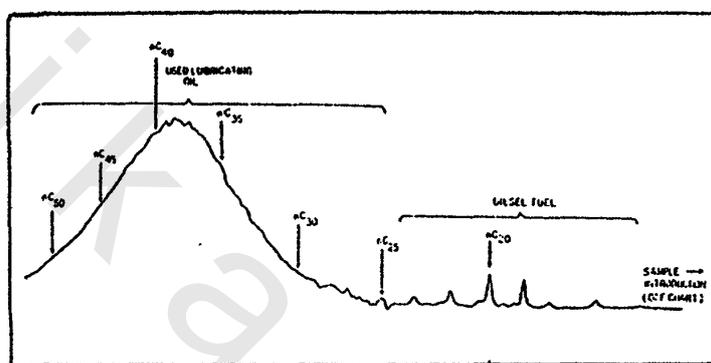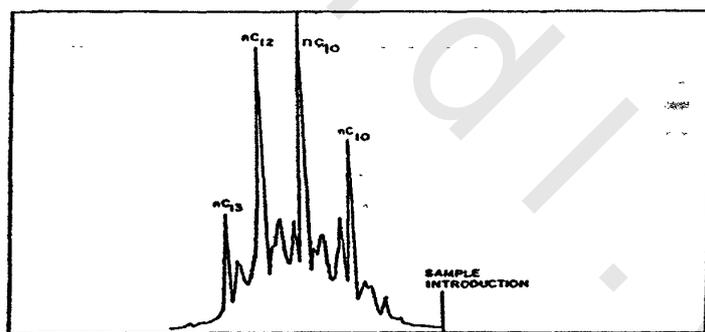

Fig.(5): Gas chromatogram for used lubricating oil.

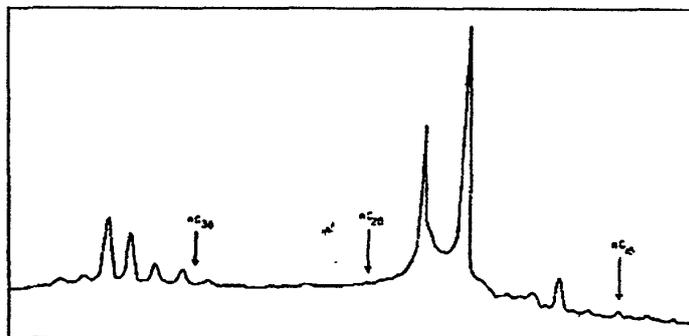**Fig.(6):** Gas chromatogram for light petroleum fraction.

Fig.(7): Chromatogram of biogenic pollutant.

Several investigators used numbers of parameters, depending on gas chromatographic analysis, which can be used for the identification of oil pollution especially concerning their origin, type and weathering degree. Among these are the following:

Pristane/Phytane ratio

The two isoprenoids pristane; Pr (2, 6, 10, 14-tetramethylpentadecane) and phytane; Ph (2, 6, 10, 14-tetramethylhexadecane) are commonly found in petroleum and its derivatives, high concentration of pristane alone can be derived from zooplankton and phytoplankton, while the presence of phytane is used as a marker compound for petroleum **(Medeiros and Bicego, 2004)**.

Carbon preference index (CPI)

This index is expressed numerically as the ratio obtained by dividing the sum of the odd carbon-numbered alkanes to the sum of the even carbon-numbered alkanes. Petroleum origin contaminants characteristically have CPI values around 1.0 **(Moustafa[b], 2004)**. Aliphatic hydrocarbons present in the marine environment are either of biogenic or anthropogenic nature **(Ahmed et al., 1998)**. Anthropogenic hydrocarbons are related to combustion / pyrolysis processes of fossil fuel, release of unburned oil products and domestic waste outfalls. Anthropogenic hydrocarbon inputs were more apparent with industrial discharges; petroleum distributors, refineries, shipping activities; dry docking and sewage outfalls **(Medeiros et al., 2005)**.

Several investigators used other parameters depending on gas chromatographic analysis for the identification of the degree of weathering; mainly biodegradation **(Barakat et al., 2002 & Tolosa et al., 2005 & De Oteyza and Grimalt, 2006)**. Among these are the following:

UCM/TRP

The ratio of unresolved complex mixture (UCM) to the total resolved peaks (TRP) is an important parameter, indicative of chronic/degraded petroleum contamination. Losses of n-alkanes and increase in UCM

(complex mixture of unseparated alicyclic, naphthenic and aromatic hydrocarbons) are indicative of weathering.

I.7.4. GC-MS spectrophotometry

Gas chromatographic techniques have now been augmented by more sophisticated analytical techniques, such as gas chromatography- mass spectrometry (GC-MS) which is a true combination of its separate parts. Detection of polycyclic aromatic hydrocarbons (PAH) is another application of this technique **(Shouming et al., 2004)**. Fortunately a subset of these compounds proved less affected by weathering than the alkanes, and there were sufficient differences in the PAH make up of various petroleum and refined products that they could serve as a fingerprinting tool **(Stephanie and Stephen, 1999)**. Mass fragmentography provides a satisfactory tool for obtaining specific fingerprints for classes and homologous series of compounds, resolved by gas chromatography. GC-MS technique has been developed for the differentiation and source identification of crude, weathered and biodegraded oils using the relative abundances of three isomeric methyldibenzothiophene compounds **(Wang and Fingas 1995)**. A data base of the ratios of the C1-DBT isomers for several hundred crude, weathered and biodegraded oils and petroleum products has been established for future fingerprinting. A rapid and simple fractionation procedure using solid-phase extraction (SPE) cartridges was developed for an accurate determination of aliphatic and polycyclic aromatic hydrocarbons in petroleum residues and further application in chemical fingerprinting by GC-MS .The SPE fractionation procedure when compared with the conventional silica-alumina adsorption chromatography show similar results but practical advantages in terms of reproducibility, analysis time, solvent reduction and cost. Moreover, is particularly suitable for routine analysis with a high sample throughput. The developed methodology was tested in characterization of contaminated oil samples collected along Spanish north-west coast, after the Prestige oil spill accident **(Alzaga et al., 2004)**. Biomarkers fingerprinting using GC-MS have historically been used by petroleum geochemists in characterization of marine oils in terms of source rock, genetic family, migration and maturation properties and in

identification of petroleum deposits. Chemical analysis of source-characteristic and environmentally persistent biomarkers generates information of great importance in determining the source of spilled oil, differentiating oils, monitoring the degradation process and weathering state of oils under a wide variety of conditions. Biomarker terpane and steranes are common constituents of crude oils, however, a few specific biomarker compounds including several geologically rare acyclic alkanes are found to exist only in certain oils and, therefore, can be used as unique markers to provide an interpretational advantage in fingerprinting sources of spilled oils and to provide additional diagnostic information on the types of organic matter that give rise to crude oil. These compounds are very resistant to biodegradation and extensive weathering of other more labile compounds left the biomarkers as the primary analytical chemistry alternative for use in fingerprinting of oils **(Albaiges and Albrecht, 1979&Wang and Fingas, 2003)**. In the past decade, use of biomarker fingerprinting techniques to study spilled oils has greatly increased, and biomarker parameters have been playing a prominent role in almost all spill work. Much of the knowledge of biomarkers and their diagnostic ratios comes from the oil geochemistry **(Peters and Moldowan 1993& Wang et al., 2004)**. A wide variety of biomarkers have been identified as being of use in characterization of crude oils and oil fractions, including tricyclic, tetracyclic and pentacyclictetrpenes, methylhopanes and steranes, methylsteranes and diasteranes. The presence of petroleum biomarkers not only serves as a clear indicator of oil contamination, but the distribution of compounds present can actually reveal the primary sources contributing to a system, as different sources may contribute distinct fingerprints. The ratio of the abundance of cyclic terpenoids to the size of the UCM further reflects the degree of weathering or degradation that petroleum residues have undergone (increase with increasing degradation or weathering). Because of the different resistance of biomarkers to biodegradation, comparisons of their relative ratios can be used to rank oils as the extent of biodegradation **(Chosson et al., 1991)**.However, it should be noted that in severely weathered or long term weathered oil, degradation of some biomarkers was observed **(Wang et al., 1999)**. Although more study is required to better understand the effect of weathering in altering the

distribution of biomarkers of petroleum contamination, more comprehensive and detailed analysis of the biomarker fingerprints of the oil would further enhance the utility of these compounds in tracing and determining the sources of petroleum contamination in the environment.

I.7.5. High performance liquid chromatography (HPLC)

It is a very useful technique in the field of analysis of polynuclear aromatic hydrocarbons (PAHs), which has been identified as the most suitable class of compounds for oil fingerprinting **(Pilli et al.,2005& Hwang and Foster, 2006)**.

High performance liquid chromatography (HPLC) is used as a separation technique for mixtures of solutes. The stationary phase may be a solid adsorbent, a liquid, an ion exchange resin, or a porous polymer, held in a metal column and the liquid mobile phase is forced through under pressure.

The advantages over other forms of liquid chromatography may be summarized:

(i) The HPLC column can be used many times without regeneration, (ii) the resolution achieved on such columns far exceeds that of the older methods, (iii) the technique is less dependent on the operator's skill, and reproducibility is greatly improved, and (iv) analysis time is much shorter. The separation and identification of PAHs in crude oils have been reported by several workers **(Todd et al., 1999)**.

HPLC system is used to measure concentrations of target semivolatile and non-volatile petroleum constituents, unlike GC systems that require complete volatilization of the sample so that it can then pass into the chromatograph, LC systems only require that the sample be dissolved in a solvent compatible with those used in the separation. The HPLC detectors most often used in petroleum environmental analysis are the fluorescence detectors. These detectors are particularly sensitive to aromatic molecules, especially the PAHs. UV detectors may be used to measure compounds

which do not fluoresce. Detection limits for PAHs by HPLC EPA Method 8310, range from 0.013-2.3 µg/L. PAHs are extracted from the sample matrix with a suitable solvent, which is then injected into the HPLC. Usually the extract must be filtered because fine particulate matter resulting in high back-pressures and eventual plugging of the column for most hydrocarbon analyses. Reverse phase HPLC (i.e., using a nonpolar column packing with a more polar mobile phase) is used the mobile phase is commonly aqueous mixtures of either acetonitrile or methanol. After the chromatographic seperation, the analytes flow through the cell of the detector. A fluorescence detector shines light of a particular wavelength (the excitation wavelength) into the cell fluorescent compounds absorb light and reemit light of other higher wavelengths (emission wavelengths). The emission wavelengths of a molecule are mainly determined by its structure. For PAHs, the emission wavelength is mainly determined by the arrangement of the rings and vary greatly between isomers. Some of these PAHs, such as phenanthrene, pyrene, and benzo(g.h.i) perylene are commonly seen in products boiling in the middle to heavy distillate range. The method uses an octadecyl column and an aqueous acetonitile mobile phase.

Crude oils from different sources can have very different polycyclic aromatic hydrocarbons (PAHs) distribution. PAHs are widespread environmental contaminants, derived from anthropogenic or natural sources **(Trabelsi and Driss 2005)**. They have toxic, mutagenic and/or carcinogenic properties. They are also highly lipid-soluble and thus readily absorbed from the gastrointestinal tract of mammals **(Samanta e al., 2002& King et al., 2004)**. They are more resistant to weathering than their saturated hydrocarbon counterparts (n-alkanes and isoprenoids) and volatile alkylbenzene compounds, thus making PAHs one of the most valuble fingerprinting classes of hydrocarbons for oil identification.

The distribution of individual PAHs of the reference 16 PAHs listed by the United States Environmental Protection Agency (USEPA). These PAHs can be divided according to the number of rings into low and high molecular weight PAHs. The low molecular weights consist of two and three aromatic rings which are 6 LPAHs while the high molecular weight consists of tetra-,

penta- and hexa-aromatic rings, they are 10 HPAHs. The nomenclatures and abbreviations of the 16 PAHs standard according to number of rings and their chemical structures are shown in Fig.(8), Four- to six-ring PAHs are highly mutagenic and carcinogenic. Two- or three-ring PAHs are less mutagenic but can be highly toxic **(Shi et al., 2005)**.

Some molecular indexes play a major role in establishing the origin of PAHs, in particular; the ratios between low and high molecular weight PAHs **(Magi et al., 2002)**, or the ratio of some isomers; e.g. phenanthrene/anthracene and flouranthene/Pyrene. They were chosen according to their thermodynamic stability **(Tolosa et al., 2004)**, among the three-ring isomers; Phe. is thermodynamically more stable and its prevalence over Ant. supports petrogenesis. Indeed, petroleum usually exhibits a quite high Phe/Ant ratio, among the four-ring isomers; Flu. is thermodynamically less stable than Pyr. Flu/Pyr>1; indicates Pyrolytic and Flu/Pyr<1 indicates petrogenic genesis of PAHs. **Baumard et al., (1998)** suggested that only the evaluation of both the ratios Phe/Ant and Flu/Pyr could prevent a misleading estimate of PAH sources. According to **De Luca et al., (2005)**; petrogenic contamination is characterized by the predominance of the LPAHs (with three and four fused rings), while the HPAHs prevail in Pyrolytic PAHs. But ratio of LPAHs: HPAHs is usually used as indices of weathering **(Bernard et al., 1996& Zakaria et al., 2001& Moustafa[b], 2004 & Hwang and Foster, 2006)**.

Low molecular weight PAHs cause acute toxicity, whereas some of the higher molecular weigt PAHs are carcinogenic. The toxicity occurs when UV excites the electrons in PAHs, resulting in the formation of toxic singlet oxygen as a by-product. The toxic singlet oxygen can damage biological membranes **(Hatch and burton 1999)**.
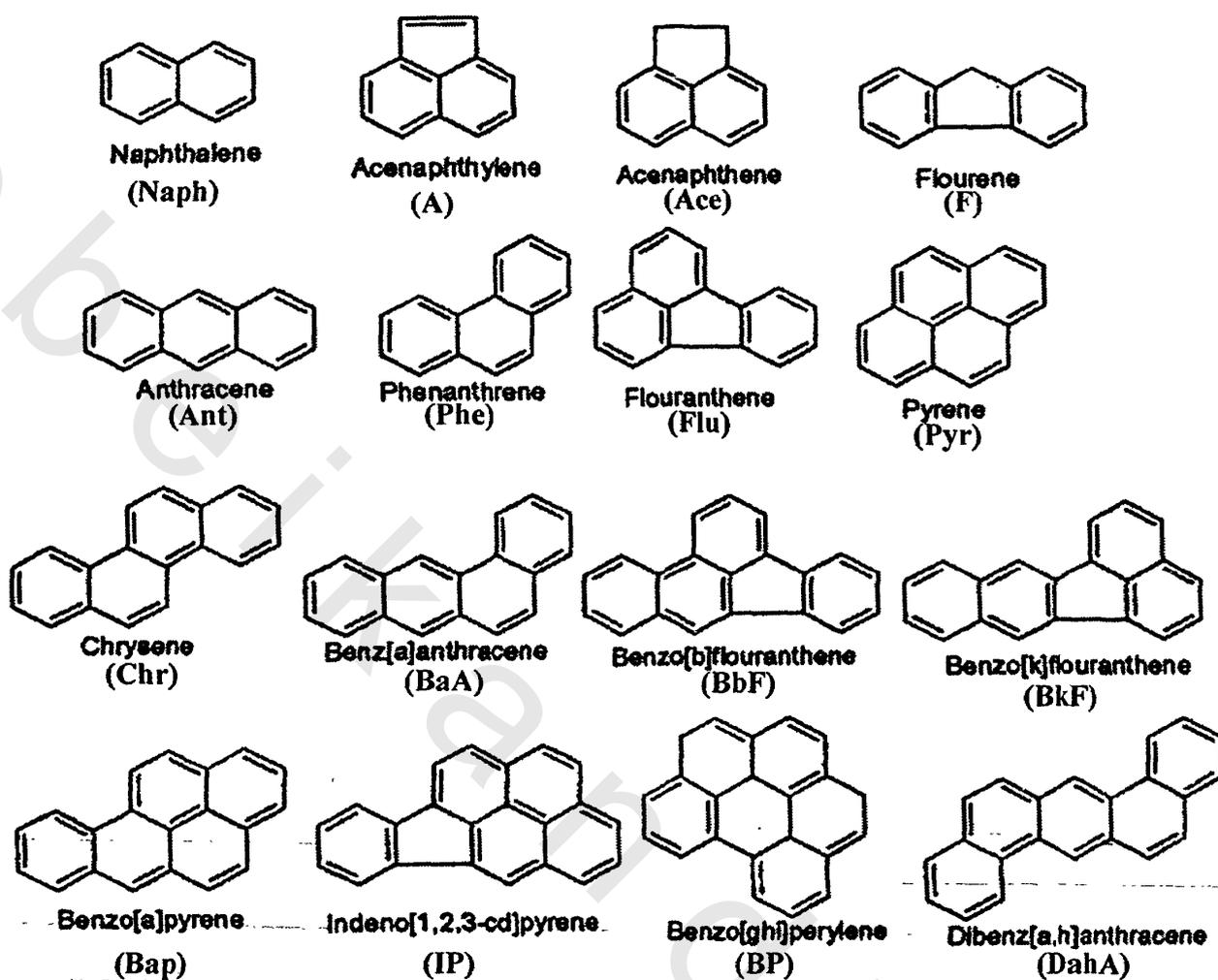
Fig.(8): The nomenclatures, chemical structure and abbreviations for 16 priority PAHs.

Investigation of (PAHs) in the aquatic environment is a very important part of environmental quality assessment that determines the status of contamination and the likely impacts it may cause to the ecosystem **(Maskaoui et al., 2002& Ibrahim, 2004).**

The PAHs contamination may result from either pyrogenic sources (incomplete combustion of organic matter, emission sources and exhausts) or from the release of petroleum into the environment **(Doong and Lin, 2004).** After entering the environment, PAHs are widely dispersed by atmospheric

transport or through stream pathways, and eventually accumulate in soil and aquatic sediments. They possess physical properties, such as low aqueous solubility and high solid-water distribution ratios, which stand against their ready microbial utilization and promote their accumulation in the solid phases of the terrestrial environment (**Johnsen et al., 2005**).

The microorganisms (naturally occurring or genetically engineered) can mineralize toxic PAHs into CO_2 and H_2O (**Samanta et al., 2002& Guo et al., 2005**).

The sources of PAHs contamination (pyrogenic and petrogenic) were achieved using both PAHs distribution and molecular indices based on ratios of selected PAH concentrations. Numerous quantitative diagnostic ratios have been defined to differentiate pyrogenic and petrogenic PAH (**Readman et al., 2002**).

I.7.6.Ultraviolet- visible spectroscopy

Crude oil spill identification and fingerprinting is one of the important applications of ultraviolet-visible spectroscopy (**Balkas et al., 1982**). It can differentiate between various oils and detect the source material of petroleum hydrocarbons (**Adlard, 1972& Lee et al., 1988**). The absorption spectra mainly reflect the composition of the aromatic compounds in the pollutant samples. It was found that the intensity ratio of absorption bands at 228 nm and over 256nm can be used as a fingerprint (**Hennig, 1979**). This ratio reflects the relative concentrations of higher molecular weight aromatic compounds in the oil and therefore remain almost unchanged after the low molecular weight aromatic compounds have been lost (**Levy, 1980**).This technique was also applied to analyze alkylbenzenes and mono and dialkyl aromatic hydrocarbons. PAHs larger than alkyl-naphthalene have strong absorption maxima at wave- length longer than 230nm (**Neff and Anderson, 1975**). The detection of aromatic components which are harmful to aquatic environment can be taken as criterion of oil pollutants contamination.

I.7.7.Infrared spectroscopy

Infrared and Fourier transform spectroscopy were used as a source identification of an oil spill **(Todd et al., 1999)**. The selection of frequencies is most beneficial to the identification and fingerprinting. The number of bands considered to be required or used for fingerprinting varies from 7 to 21 bands. Weathering was monitored by noting 18-21 peaks in the spectrum in the range 1300cm-1 to 1700 cm-1 **(Todd et al., 1999)**. Some distinctive bands have disappeared, others became of lower intensity as weathering proceed **(Abu-Elgheit, 1980)**. Various statistical methods and pattern-recognition techniques have been reported in order to improve both the capability and reliability of fingerprinting and/or classification **(Todd et al., 1999)**. A detailed comparison of selected absorbance bands or ratios of them between unknown and suspected crude oil samples was reported. It was found that the absorbance values of the bands present when ratioed to the 2960, 1750, 1375 and 720 cm-1, at least one ratio will stand out as being distinctive for specific oil. There are eleven ratios, Table (1) that would help to distinguish between various oils. For unidentified oil, all 11 ratios must be calculated and compared to the ratios of reference crude oils, to determine the identity of the oil **(Frank et al., 1979)**.

Table (1): IR Significant Ratios

2960 cm^{-1}	1750 cm^{-1}	1375 cm^{-1}	720 cm^{-1}
3700/2960	1540/1750	1600/1375	860/720
1780/2960		810/1375	810/720
1750/2960		720/1375	
1700/2960			
860/2960			

For IR-based methods, TPH is defined as extractable matter by a solvent (Freon 113), which is not removed by silica gel and can be detected by IR at a specified wavelength. The primary advantage of IR-based TPH methods is that they are simple, quick and inexpensive. Detection limits for a commonly used IR-based TPH method, EPA Method 418.1, are approximately 1 mg/L in water and 10 mg/kg in soil. This TPH method often

suffers from poor accuracy and precision, especially for heterogeneous soil samples. IR-based methods give no information on the type of fuel present, no information about the presence or absence of toxic molecules, and no specific information about potential risk associated with the contamination.

Most IR-based methods in the United States typically measure the absorbance at a single frequency (usually 2930 cm-1) which corresponds to the stretching of aliphatic CH2 groups. Some methods, especially in Europe, use multiple frequencies including 2960cm-1 for CH3 groups and 2900 to 3000 cm-1 for aromatic C-H bonds.

I.7.8.Statistical and numerical analysis

The quantitative oil analysis data, when used in conjunction with multivariate statistical analyses, can increase the precision of analytical data and provide an unbiased and defensible means to differentiate among qualitatively similar oils. In modern oil investigations, various statistical and numerical analysis techniques have been developed and applied to the data interpretation.These powerful tools include principal components analysis (PCA), discriminate analysis and cluster analysis. Among them, PCA is the most widely used and powerful multivariate analysis technique. It is used to transform original sample composition data into new, smaller and uncorrected variables called principle components. PCA is now useful tool for analyzing the structure of the data and reducing the dimensionality of the pattern vectors **(Stout et al 2001 & Colombo et al., 2005)**. PCA was carried out on the metals, organic carbon and particles to cluster the similarities of the variables so as to establish the predicted or adjusted environmental monitoring program (EMP) rating **(Chou et al., 2004)**. The application of sophisticated statistical analysis techniques to oil analysis is a relatively new and dynamic area of research which enhances the interpretive power of petroleum hydrocarbon fingerprinting and promises to greatly improve the identification of oil spill sources.

I.8.Bioremediation

Bioremediation is of interest worldwide as a potential oil spill clean up technique under certain geographic and climatic conditions. It is a technology that offers great promise in converting the toxic compounds of oil to non-toxic products without further disruption of the local environment **(Chaîneau et al., 2005)**. Microbiologists have studied this process since the 1940,s **(Zobell, 1946)**. Bioremediation is a proven alternative treatment tool that can be used in certain oil-contaminated environments. Typically, it is used as a polishing step after conventional mechanical clean-up options have been applied.

Bioremediation has numerous applications, including clean-up of ground water, soils lagoons, sludges, and process-waste streams. It can be also an effective technology to remove petroleum hydrocarbons in oil-contaminated sediments of the inter-tidal marine environment.

Bioremediation has been used on very large-scale application, as demonstrated by the shore-line clean-up efforts in Prince William Sound, Alaska, after the Exxon oil spill. Although the Alaska oil-spill clean-up represents the most extensive use of bioremediation, there has been much other successful application on smaller scale.

Biodegradation is a naturally occurring process, where indigenous oil-degrading microorganisms are capable, under favorable conditions, of utilizing petroleum hydrocarbons as a metabolic carbon source. Although rapid growth of the indigenous microbial biomass can occur in beach sediments following oil contamination, the subsequent depletion of nutrients and readily degradable organic carbon can rapidly limit biodegradation.

The two main approaches to oil-spill bioremediation are: (1) bioaugmentation, in which oil-degrading microorganisms are added to supplement the existing microbial population, and (2) boistimulation, in which nutrients, or other growth limiting substances, are added to stimulate

the growth of indigenous oil degraders **(Trindad et al., 2005 & Olaniran et al., 2006)**.

Researches are on going to evaluate bioremediation and phytoremediation (plant-assisted enhancement of oil biodegradation) for their applicability to clean up oil spills contaminating salt marshes and freshwater wetlands **(Lin and Mendelssohn, 1998& Corgie et al., 2004 & Sun et al., 2004)**.

Bioremediation is an attractive approach of cleaning up petroleum hydrocarbons because it has the following advantages **(Bento et al., 2004)**;

- It may result in complete degradation of toxic organic compounds to nontoxic byproducts.
- There are minimum mechanical equipment requirements.
- It can be implemented as in-situ or ex-situ process. In-situ bioremediation is safer since it does not require excavation of] contaminated soils. Also, it does not disturb the natural surroundings of the site.
- Low cost compared to other remediation technologies.
- It is very safe because it relies on microbes that naturally occur in the soil, and/or the contaminated sites, no dangerous chemicals are used in bioremediation, it also prevents the release of harmful gases into the air.

But bioremediation has the following disadvantages **(Bento et al., 2004)**;

- There is a potential for partial degradation to metabolites that are still toxic and/or potentially more highly mobile in the environment.
- The process is highly sensitive to toxins and environmental conditions.
- Extensive monitoring is required to determine biodegradation rates.
- It may be difficult to control volatile organic compounds during ex-situ bioremediation process.
- Generally requires longer treatment time as compared to other remediation technologies.

The biggest challenge facing the responder is maintaining the proper conditions for maximal biodegradation to take place, as one of the difficulties of developing bioremediation strategies lies in achieving as good or better results in the field as in the laboratory. Several factors influence the success of bioremediation, the most important being the type of microorganisms present at the site, the physical and chemical characteristics of the oil, the oil surface area, nutritional requirements (carbon, nitrogen and phosphorus), some other environmental factors; aeration, water content, pressure, pH, salinity, temperature and also the microbita also affect the rate of biodegradation **(Trindad et al., 2005)**.

In almost all cases, the presence of oxygen is essential for effective biodegradation of oil. Anaerobic decomposition of petroleum hydrocarbons leads to extremely low rates of degradation **(Frankenberger 1992)**. The aerobic biodegradation of PAHs is well documented **(Beckles et al., 1998& Lei et al., 2005)** while anaerobic biodegradation has received relatively little attention. Earlier studies indicate that PAHs were persistent in soil or sediment matrices in the absence of oxygen **(Vidali, 2001)**. With recent reports on the capability of bacteria to biodegrade PAHs under various redox conditions, especially those with lower numbers of benzene rings, bioremediation had become a very promising concept for the restoration of PAHs contamination **(Lei et al., 2005)**. Some anaerobic metabolites of PAHs have been identified in oil spill and petroleum reservoir environments **(Huang et al., 2004)**.

The ideal pH range to promote biodegradation is close to neutral (6-8). For most species, the optimal pH is slightly alkaline, that is, greater than 7 **(Lee and Saunders, 2003)**. The moisture content of the contaminated soil will affect biodegradation of oils due to dissolution of the residual compounds, dispersive actions, and the need for microbial metabolism to sustain high activity. The moisture content in soil affects microbial locomotion, solute diffusion, substrate supply, and the removal of metabolic by-products. Excessive moisture will limit the gaseous supply of oxygen for enhanced decomposition of petroleum hydrocarbons. Most studies indicate that optimum moisture content is within 50-70% of the water holding

capacity (**Frankenberger, 1992**). All biological transformations are affected by temperature. Generally, as the temperature increases, biological activity tends to increase up to a temperature where enzyme denaturation occurs. The presence of oil should increase soil temperature, particularly at the surface. The darker color increases the heat capacity by adsorbing more radiation. Generally, the optimal temperature for biodegradation to occur ranges from $18^{\circ}C$ to $30^{\circ}C$. Minimum rates would be expected at $5^{\circ}C$ or lower (**Frankenberger 1992 & Coulon et al., 2004**). There are at least 11 essential macronutrient and micronutrient elements that must be present in the soil in proper amounts, forms, and ratios to sustain microbe growth. These 11 elements are nitrogen, phosphorus, potassium, sodium, sulfur, calcium, magnesium, iron, manganese, zinc, and copper. Nitrogen is usually the main limiting nutrient governing the rate of decomposition of petroleum hydrocarbons. However, small amounts of phosphorus fertilizers may also be necessary to stimulate biodegradation (**Chaîneau et al., 2005**). Biodegradation rates in soils are also affected by the volume of product released to the environment. At concentrations of 0.5 - 1% of oil by volume, the degradation rate in soil is fairly independent of oil concentrations. However, as oil concentration rises, the first order degradation rate decreases and the oil degradation half-life increases. Ultimately, when the oil reaches saturation conditions in the soil (i.e., 30-50% oil), biodegradation virtually ceases.

Degradation of aromatic hydrocarbons, such as toluene, can yield phenolic and benzoic acid intermediates. Various microbial populations may be inhibited by compounds such as phenol and toluene, particularly at high concentrations. Although phenol- and toluene-degrading microorganisms have been isolated in soil exposed to low concentrations of these compounds, they are biocidal at elevated concentrations (**Frankenberger, 1992**). The inhibitory effects of heavy metals can also influence biodegradation of organic materials (**Sandrin and Maier, 2003**). The presence of heavy metals in oil sludge, motor oil, and used crankcase oil may have deleterious effects on the hydrocarbon oxidizers in decomposing petroleum hydrocarbons (**Frankenberger, 1992**). Other elements of concern include zinc, copper, chromium, nickel, and cadmium. With repeated applications of oily sludge to

a land farm operation, heavy metals may accumulate at levels in which biodegradation may be reduced.

Hydrocarbons generally differ in their susceptibility to microbial attack and have generally been ranked in the following order of decreasing susceptibility: n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes > light aromatics > high-molecular-aromatics > polar compounds.

To distinguish between oil lost by physical means and oil that has been biodegraded, biodegradable constituents are normalized to a resistant biomarker compound eg. pristane, phytane and hopanes. In refined oils that have no hopanes, biodegradation can be confirmed by normalizing to a highly substituted 4-ring PAH or by examining the relative rates of disappearance of alkanes and PAH homologs **(Huang et al., 2004).**

The ability to degrade hydrocarbon components of crude oil is widespread among marine bacteria **(Floodgate, 1995)** and bioremediation has proven to be an effective method for cleaning up residual oil in a variety of coastal environments, such as rocky shore lines, pebble and coarse sand beaches **(Atlas and Bartha, 1972 & Roling et al., 2004).** Due to the high carbon content of oil and the low levels of other nutrients essential for microbial growth, treatment of beached oil with phosphorous and nitrogen is generally required to enhance the growth of hydrocarbon-degrading bacteria and to stimulate oil degradation **(Roling et al., 2004).**

Measuring the success of bioremediation of oil spills is based on several parameters, among them the degradation of PAHs in crude oil.

Harnessing natural microbial processes to clean up PAH-contaminated soils is a promising approach to reduce the amount and toxicity of PAHs on waste sites. However, developing a bioremediation technology the can degrade PAHs of all shapes and sizes is difficult because PAHs differ greatly in their degree of biodegradability. Typically, the greater the number of fused rings the more resistant the compounds are to biodegradation. Despite these

challenges, many scientists believe it is possible to develop strategies for utilizing microbes to remove PAH mixtures from contaminated soils. A greater understanding of the kinds of microbes that can degrade the larger PAHs, and the combinations of microbes that result in the most efficient and extensive degradation of these contaminants is needed to develop effective bioremediation strategies for PAH mixtures (**Ravelet et al., 2000 & Gaskin and Bentham, 2005**).

For optimum biodegradation conditions, it is important to know the characteristics of the contaminated site before beginning treatments. Basic information such as residual oil concentration, population density of the oil-degrading microorganisms and the biodegradation potential, are key factors to be considered for bioremediation of oil-polluted sites. In most field studies, enhancing biodegradation of petroleum hydrocarbons depends on the specific microbial population present. The composition of the microbial population is affected by the environmental conditions and the composition of the hydrocarbons.

In this study, a comparative evaluation of biostimulation and bioaugmentation for bioremediation of hydrocarbon contaminated sediments with special emphasis on PAHs present in the contaminated sediments will be performed.

I.9.Environment of the studied area

I.9.1.Suez Canal

Suez Canal is actually the first canal linking the Mediterranean Sea to Red Sea. It covers an area of about 4000km2 located in the Northeastern part of Egypt, approximately between latitudes 29° 35 - 31° 20 N and longtitudes 32° 05 - 32° 40 E. It is bounded Northwards by the Mediterranean Sea and Southwards by Gebel Khashm El Galala.

The Suez Canal zone is covered by sedimentary rocks which belong to the cretaceous, Middle Eocene, Late Eocene, oligocene, Early Miocene,

Middle Miocene, Late Miocene and Pleistocene-Holocene ages. The study of these sediments is based on multispectral aerial and normal aerial photographs, infrared thermal images and ground geological mapping.

Sand dunes of different types and sizes are covering considerable areas in the Eastern part of the investigated Suez Canal zone and around marshes neart El Devreswar, El Ismailiya and El Qantara. At a distance of about 3 to 5 km to the east of the Suez Canal, sand dunes of the barchan type cover the topographically irregular area, which extends Northwords to near El Qantara El Sharkiya. The sand dunes of this type, assuming various sizes have mostly migrated on the crests and slopes of this undulated area. The dunes are generally of low elevation and surrounded by eolian accumulations.

The Suez Canal zone is characterized by the presence of extensive water surface represented mainly by the Gulf of Suez, Bitter Lakes, El Temsah Lake and El Manzala Lake. The volume of the Suez Canal effluent entering the Mediterranean was computed to reach 2 x 109 m3/mo, indicating that the southeast Mediterranean is highly affected by effluent disposal from the Suez Canal.

The Great and Little Bitter Lakes are nearly elongated in shape and they are connected with each other. The Great Bitter Lake extends approximately North West - South East El Temsah Lake has nearly triangular shape with elongated sides extending roughly East - West. There are two lagoons connected with the Little Bitter Lake at its eastern shore, one almost at the center of the shore and the second near the connection between little and Great Bitter Lakes.

It is the longest Canal in the world with no locks. Navigation goes day and night. Compared with other waterways, percentage of accidents is relatively small.

The need to analyze for petroleum hydrocarbons in contaminated sediments may arise for different reasons, (a) Because of the desire to establish the nature and the level of oil polluting the studied area, (b) To

assess the effect of the different petroleum activities on the surrounding marine environment, (c) To know how wide spread is the oil and to what extent it has affected marine life, (d) It can provide the result of atmospheric fallout over time.

Numerous analytical methods have been described on how to detect and estimate hydrocarbons contaminating sediments. Some of these methods can be used as "fingerprints" to determine the origin of these hydrocarbons. Other suggests the composition of oil polluting the sediments. The concentrations for the extracted oils from surface sediment samples give information about the level of oil pollution in the studied sites. The hydrocarbon contents for oil incorporated to sediments may depend on the nature of each area, the presence or absence or near by petroleum activities and its types, the different environmental conditions, transport of sediments by currents, turbidity flows, uptake and release by organisms, biochemical and photochemical oxidation, and the presence of other compounds capable of solubilizing the hydrocarbons in the water column.

I.9.2. Temsah Lake

Temsah Lake, one of the water bodies that constitute the Bitter Lakes located North of Suez Canal is a land engulfed embayment with a total area of 15 km2. Temsah Lake receives high salinity water from the Suez Canal, mainly from the south, and freshwater from a Nile canal and other sources **(El-Serehy and Sleigh, 1992)**. This lake supports fishing and tourism that employ a large number of local citizens and provide a significant portion of the district revenues. Temsah Lake is the main wet dock of the city, a small port that also supports a variety of marine works, including maintenance work of the Suez Canal Authority and its allied maritime works **(Tundo et al., 2005)**. The quality of life, contamination level and its source in the lake has been a major concern for the local authorities for the last few years because of the noticed sharp decline in the biodiversity and fish quality harvested from the lake **(Tundo et al., 2005)**. Temsah Lake is exposed to a variety of aliphatic and aromatic hydrocarbons that originate from shipping

activities, ballasting water, maintenance and maritime works in the several clocks around **(Tundo et al., 2005)**.

Several reports have rerecorded the contamination level of a variety of pollutants in Temsah lake and its marine organisms, including dioxins **(Tundo et al., 2004 & 2005)** chlorinated hydrocarbons, polychlorinated biphenyl and PAHs **(Ahmed et al., 2001 & Said and Agroudy, 2006)**.

I.9.3. Gulf of Suez

The Gulf of Suez (GOS) extends for about 250km South-Southeast from the Suez port in the north (Lat. 29o 56') to Shadwan Island in the south (Lat. 27o 36'). The width of the gulf varies between 20 and 40km, and its depth throughout its axis is fairly constant with a mean of 45m **(El-Sabh and Beltagy, 1983)**. Depth increases abruptly to about 250m at its mouth. The Northern part of the Gulf of Suez (Suez Bay) is characterized by anticlockwise water circulation **(Meshal, 1970 & Soliman, 1996)**, and it is an eutrophic region **(Hamed, 1992)**. The Suez Bay ecosystem is suffering of adverse effects of sewage discharge as well as industrial wastes from various activities based in Suez City (as oil refineries, fertilizer and chemical industry, power stations and harbours).

Additional environmental threats might arise from envisaged development plants. Four hundred fifty new projects, e.g. steel factory, power station, pipelines, marinas, touristic village, vegetable oil industries,...etc. are planned for the Northern part of the Gulf of Suez. As aresult of industrialization, gulf coastal areas are increasingly subjected to pollution with subsequent threaten of human health **(El-Moselhy and Gabal, 2004)**.

The Gulf of Suez, Egypt, is nationally and internationally recognized as a principal attraction for tourists, marine biologists and divers for its coral reefs and excellent underwater aquatic life. Pollution of the marine environment in the GOS represents one of the most serious environmental problems confronting the Egyptian Government. Petroleum hydrocarbons are potentially the most likely source of pollutants **(Barakat et al., 2002)**.

Coastal oil pollution of the Gulf of Suez is a serious problem. The principal sources are anthropogenic and they include,(a) offshore oil production mostly in the Southern regions;(b) wastewater effluents from the industrial activities in the Northern region which comprise oil refineries, fertilizer plants and petrochemical industries; (c) ballast and/or bilge water from tankers and large vessels awaiting berth or those in transit through the Suez Canal; (d) sewage and domestic waste from Suez City; (e)accidental oil spill and leakage; (f) tanker operations at Sumed pipeline terminal in Ain Sokhna; and (g) atmospheric deposition **(Ibrahim, 2004)**.

I.9.4. Samples locations and collection

Sampling from the environment is one of the most difficult sampling processes. Often, the most careful analysis is invalidated by inadequate or improper sampling. The sampling process for any type of environmental analysis differs markedly from any more common problems of sampling and analysis. There are several bases for this statement. First, there is no defined limit on the environment. Second, it is hardly ever uniform and there are rarely sharp boundaries of no uniformity. Finally, essentially every component is in trace quantity. It can be lost or destroyed in the sampling process by adhering to the container walls, reacting with other components of the sample, or reacting with elements of sampling system. Protection of the collected samples against chemical and physical changes during transit or storage is essential to ensure sample integrity. The collection procedures used should assure that no contamination of the samples occurs.

I.9.4.1. Samples locations

All these samples were collected from three locations in August 2005 for this study. The types and geographic locations of these sites are represented in Tables (2, 3, 4) and Figs.(9,10,11).

I-Suez Canal samples

Five surface water and five surface sediment were collected from different locations.

II-Temsah Lake samples

Eleven surface water, eight surface sediment and four fish samples were collected from different locations all over the Temsah Lake.

III-Gulf of Suez samples

Seven surface water and seven surface sediment were collected from different locations.

I.9.4.2. Samples collections

All the collected samples were stored in refrigerator at 4oC until analysis **(Readman et al., 2002)**. Extraction began as soon as possible after collection to avoid microbial degradation.

Surface water samples were collected using narrow neck borosilicate glass bottles with Teflon lined caps. The samples were acidified to pH2 using 10% HCl to preserve them against bacterial action during transportation and storage.

Surface sediment (0-2cm) samples were collected using a Van Veen grab sampler and stored in cleaned glass jars.

Fish samples were collected from different sites to represent various zones of territorial aquatic area of Temsah Lake wrapped in clean aluminum foil.
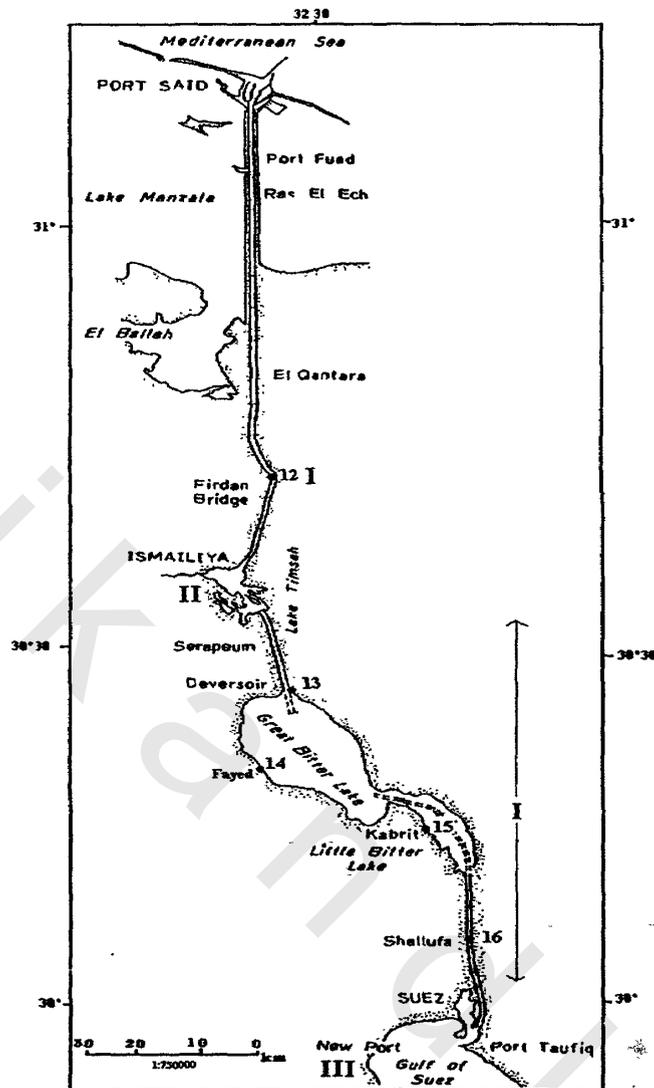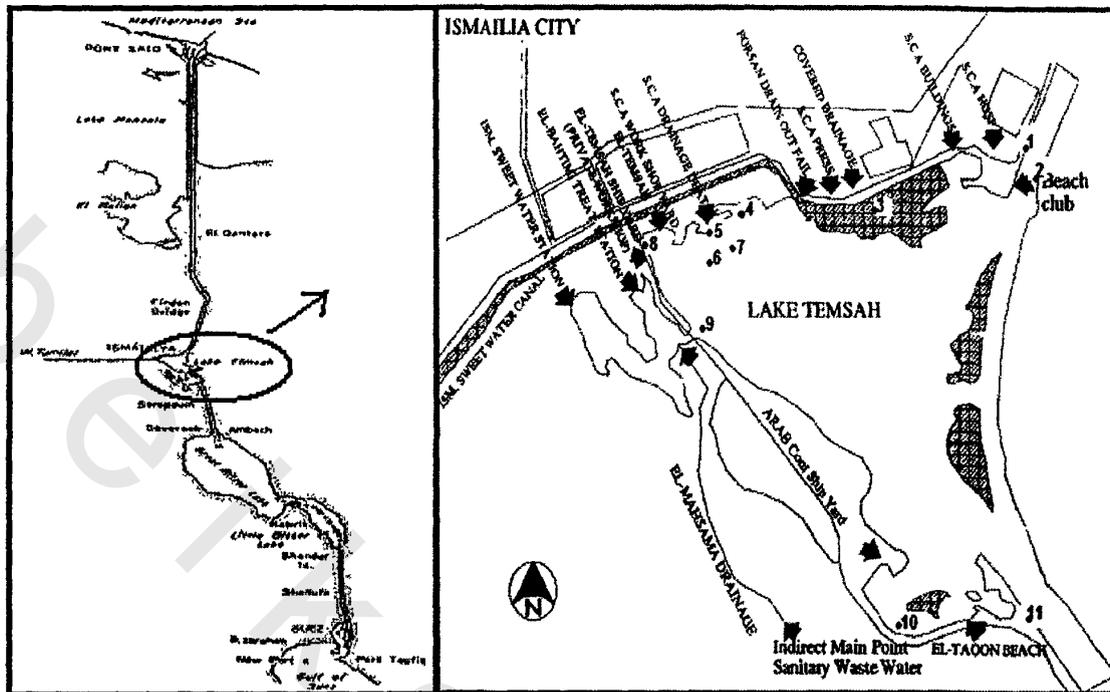
Fig.(9): Map of Suez Canal showing the studied locations

Table (2): Sampling sites of location-I

Sites	Type of samples	
	Water	Sediment
I-12	W12	S12
I-13	W13	S13
1-14	W14	S14
I-15	W15	S15
I-16	W16	S16

Fig.(10): Map of Temsah Lake showing the studied locations

Table (3): Sampling sites of location-II

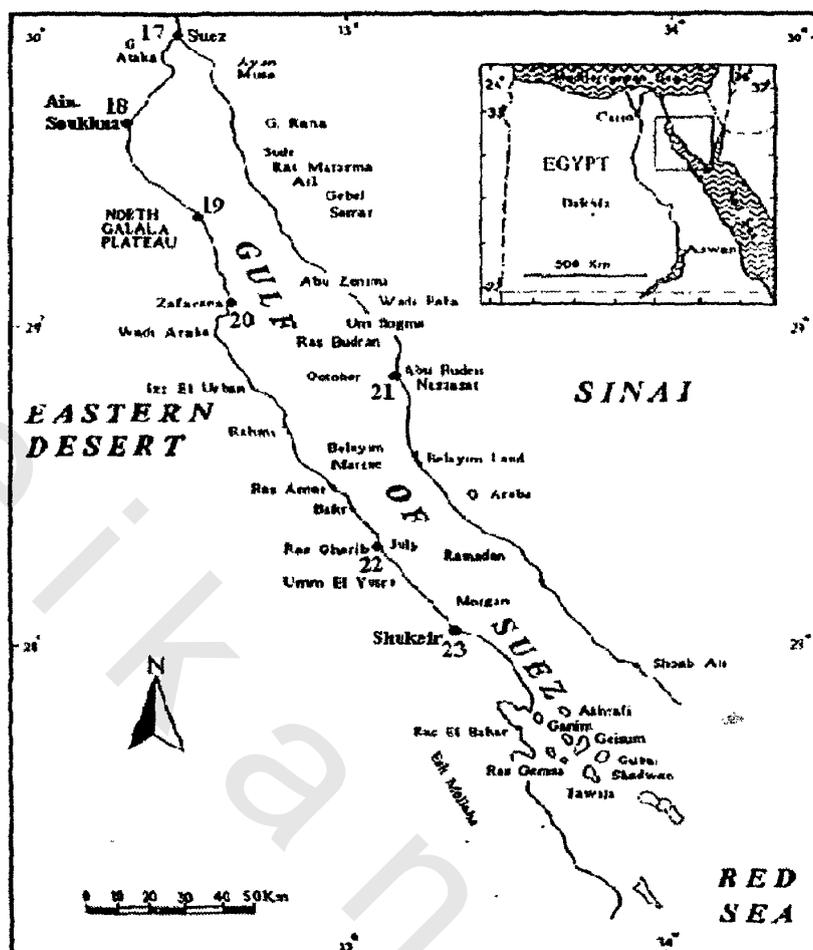| Sites	Type of samples		
	Water	**Sediment**	**Fish**
II-1	W1	S1	F1
II-2	W2	Rock area	Not represented
II-3	W3	S3	F3
II-4	W4	S4	Not represented
II-5	W5	Rock area	Not represented
II-6	W6	Rock area	Not represented
II-7	W7	S7	Not represented
II-8	W8	S8	Not represented
II-9	W9	S9	F9
II-10	W10	S10	Not represented
II-11	W11	S11	F11

Fig.(11): Map of Gulf of Suez showing the studied locations

Table (4): Sampling sites of location-III

Sites	Type of samples	
	Water	Sediment
III-17	W17	S17
III-18	W18	S18
III-19	W19	S19
III-20	W20	S20
III-21	W21	S21
III-22	W22	S22
III-23	W23	S23