Zur Homepage der Dissertation

Environmental Organic Geochemistry of Sediments from Wadi Gaza and Investigation of Bioremediation of Petroleum Derivatives and Herbicides by Cyanobacterial Mats under Different Experimental Conditions

Von der
Fakultät für Mathematik und Naturwissenschaften
der
Carl von Ossietzky Universität Oldenburg
zur Erlangung des Grades und Titels eines

Doktors der Naturwissenschaften
-Dr. rer. nat.-

angenommene

Dissertation

von Herrn

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Tag der Disputation:	05.03.2004

For my parents

and

my wife, my children,

my brothers and my sister

Publications and Presentations

Safi, N. M. D., Lee, N., Köster, J., Safi, J., El-Nahhal, Y., Wagner, M., Rullkötter, J., 2003. Biodegradation of petroleum model compounds by cyanobacterial mats in mesocosm experiments in Gaza (Palestine) (Oral). In: Book of abstracts I, 21st International Meeting on Organic Geochemistry, September 8-12th, pp.138-139, Krakow, Poland.

Safi, N. M. D., Köster, J., Safi, J., El-Nahhal, Y., Rullkötter, J., 2002. Biodegradation of polycyclic aromatic petroleum compounds by cyanobacterial mats in mesocosm experiments in Gaza (Palestine) (Oral). In: Book of Abstracts, 3rd European Meeting on Environmental Chemistry, December 11-14, p. 4, Geneva, Switzerland.

Köster, J., Groetzschel, S., Safi, N., de Beer, D., Rullkötter, J., 2002. Laboratory experiments with microbial mats: Biodegradation of petroleum model compounds and physiological response to pollution (Poster). In: Book of Abstracts, 3rd European Meeting on Environmental Chemistry, December 11-14, p. 110, Geneva, Switzerland.

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Acknowledgments

I would like to express my deep gratitude and many thanks to my supervisor Prof. Dr. Jürgen Rullkötter for giving me the opportunity to carry out this Ph.D. work and for his advice, innovative ideas, interesting discussions, continuous encouragement through all my research and the excellent working environment at the Institute of Chemistry and Biology of the Marine Environment (ICBM). Special thanks are also due to Prof. Dr. Gerd Liebezeit for being a referee of this thesis.

I am indebted to Dr. Jürgen Köster for his continuous support, encouragement, critical reading, comments and suggestions. Dr. Köster was always available for advice and motivating discussions.

My sincerest thanks go to Prof. Dr. Jamal Safi and Dr. Yasser El-Nahhal at the Environmental Protection and Research Institute (EPRI), Gaza, Palestine for their advice, continuous encouragement and supplying the samples through all my study.

Many thanks are due to the members of the Organic Geochemistry group at (ICBM) for their general help, comfortable atmosphere in our labs, and for constructive comments, stimulating discussions and ideas.

I thank Dr. Dirk de Beer for giving me the opportunity to carry out experiments at the Max Planck Institute for Marine Microbiology (MPI), Bremen.

This research was funded by the Deutsche Forschungsgemeinschaft (DFG), grant no. Ru 458/18, and by ICBM.

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Abbreviations

AHC:	Total aliphatic hydrocarbons.
Aqu:	Sum of *n*-alkanes from aquatic organisms: pentadecane, heptadecane and nonadecane
BTMA	Benzyl-trimethylammonium
CEC:	Cation exchange capacity
CPI:	Carbon preference index
DBT:	Dibenzothiophene
2,4-D:	2,4-Dichlorophenoxyacetic acid
Dmphen**:**	Dimethylphenanthrene
Dt_{50}:	Degradation half-life time
EOM:	Extractable organic matter
EPRI:	Environmental Protection and Research Institute
Etphen:	Ethylphenanthrene
FA:	Fatty acids
FID:	Flame ionisation detector
GC:	Gas chromatography
GC/MS:	Gas chromatography/mass spectrometry
ICBM:	Institute of Chemistry and Biology of the Marine Environment
IS:	Internal standards
K:	Degradation rate constant
MDBT:	Methyldibenzothiophene
MDI:	Methyldibenzothiophene index
mg:	Milligram
µg:	Microgram
Mphen:	Methylphenanthrenes
MPLC:	Medium Pressure Liquid Chromatography
MSTFA:	N-methyl-N-trimethylsilyltrifluoroacetamide
NA:	*n*-Alkanes
NOCs:	Non-ionic organic compounds
NSO:	Polar heterocompounds (nitrogen-, sulfur-, oxygen)
OCC:	Organo-clay complex
PAHs:	Polycyclic aromatic hydrocarbons
Ph:	Phytane

Phen:	Phenanthrene
Pr:	Pristane
r^2:	Correlation coefficient
SLCF:	Sum of the liquid chromatography fractions
TCA:	Tricarboxlic acid
Ter:	Sum of terrestrial *n*-alkanes: heptacosane, nonacosane and hentriacontane
UCM:	Unresolved complex mixture

Abstract

This thesis presents for the first time results of (i) an organic geochemical analysis of sediments from Wadi Gaza (Gaza Strip, Palestine) and their level of pollution, particularly with petroleum and petroleum derivatives, and (ii) an investigation of the degradation potential of naturally occurring cyanobacterial mats originating from the chronically oil-polluted environment of Wadi Gaza. The degradation of petroleum model compounds is analyzed in laboratory and mesocosm experiments involving Egyptian crude oil and 2,4-dichlorophenoxyacetic acid and terbutryn herbicides in laboratory experiments.

The organic composition of extractable lipids in the sediments indicates that Wadi Gaza receives a moderate supply of anthropogenic material. Using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), aliphatic hydrocarbons from petroleum sources were identified in the Wadi Gaza sediments. The presence of aliphatic isoprenoid alkanes (pristane and phytane), the presence of an unresolved complex mixture (UCM), the UCM/*n*-alkane ratio, the low carbon preference index (CPI ca. 1) and the presence of petroleum biomarkers such as steranes and hopanes indicate the presence of petroleum-related hydrocarbons. The biomarker parameters based on hopanes and steranes reveal significant differences between the sediment samples, which suggest that there are multiple sources of oil or refinery products. In the aromatic hydrocarbon fraction, the petroleum origin was indicated by the presence of phenanthrene and its alkylated homologues as well as that of dibenzothiophene and its alkylated homologues. The ratio of methylphenanthrenes to phenanthrene shows that the sediments are dominated by fossil-fuel derived phenanthrenes. The methyldibenzothiophene index (MDI) of Wadi Gaza sediments confirms the input of crude oil and petroleum derivatives. Compounds deriving from terrestrial sources (higher plants), such as long-chain *n*-alkanes, *n*-fatty acids and *n*-alcohols, and marine-derived hydrocarbons were also present.

Wadi Gaza cyanobacterial mats effectively degraded both aliphatic (pristane and *n*-octadecane) and aromatic compounds (phenanthrene and dibenzothiophene) in both laboratory and mesocosm experiments. Degradation of the model compounds in the laboratory experiment was faster than in the mesocosm. The degradation rates depend on the type of compound, aromatic compounds were degraded faster than aliphatic hydrocarbons.

In the crude oil experiment, the mats efficiently degraded both *n*-alkanes and the isoprenoids pristane and phytane. The *n*-C_{14-20} alkanes were degraded most rapidly, the *n*-C_{21-27} alkanes were degraded the slowest, and the *n*-C_{28-35} alkanes were degraded at intermediate

rates. Pristane and phytane; were degraded 94 and 88%, respectively within 60 days.

The mats had a high capacity for herbicide degradation; more than 80% of 2,4-dichlorophenoxyacetic acid (2,4-D) were removed both in the light and in the dark within 30 days. Terbutryn was degraded to 28% and 36% of its original concentration in the light and in the dark, respectively, within 80 days. The observed half-life times of 49 and 56 days in the terbutryn experiment indicate that fast degradation of terbutryn occurred by the Wadi Gaza cyanobacterial mat organisms.

We conclude that the Wadi Gaza cyanobacterial mats, are heavily polluted by crude oil and petroleum derivatives, and that the potential for bioremediation by degrading petroleum derivatives and herbicides is strong.

Kurzfassung

Diese Doktorarbeit präsentiert erstmalig Ergebnisse von 1.) organisch-geochemischen Untersuchungen an Sedimenten aus dem Wadi Gaza und der Analyse ihres Verschmutzungsgrades, besonders mit Erdöl und Erdölderivaten, und 2.) Untersuchungen zum Abbaupotential natürlicher Cyanobakterienmatten, die in der chronisch mit Öl verschmutzten Umgebung des Wadi Gaza vorkommen. Untersucht wurde der Abbau von Erdöl-Modellverbindungen in Labor- und Mesokosmos-Experimenten sowie von ägyptischem Rohöl und der Herbizide 2,4-Dichlorophenoxyessigsäure und Terbutryn in Labor-Experimenten.

Die Zusammensetzung der extrahierbaren Lipide der Sedimente zeigt, dass das Wadi Gaza eine mäßig starke Zufuhr von anthropogenen Verunreinigungen erfährt. Mit Methoden der Gaschromatographie und Gaschromatographie-Massenspektrometrie wurden erdölbürtige aliphatische Kohlenwasserstoffe identifiziert. Die Anwesenheit von Isoprenoidalkanen (Pristan und Phytan), das Vorkommen von chromatographisch unaufgelösten, komplexen Kohlenwasserstoffgemischen (*unresolved complex mixtures*, UCM), das UCM/*n*-Alkan-Verhältnis, das Fehlen einer Bevorzugung von *n*-Alkanen bestimmter Kettenlänge (*carbon preference index*, ca. 1) und das Auftreten von Erdöl-Biomarkern wie Steranen und Hopanen zeigen eine Verschmutzung mit Erdöl an. Biomarker-Parameter von Steranen und Hopanen lassen deutliche Unterschiede zwischen den untersuchten Sedimenten erkennen, die auf eine Herkunft der Öle oder Raffinerie-Produkte aus mehreren verschiedenen Quellen hinweisen. In der Aromaten-Fraktion wird die Herkunft aus Erdöl durch das Vorkommen von Phenanthren und Dibenzothiophen und deren alkylierten Homologen angezeigt. Das hohe Verhältnis von Methylphenanthrenen zu Phenanthren zeigt, dass diese Verbindungen in den Sedimenten vorwiegend aus fossilen Kohlenwasserstoffen stammen. Auch der Methylbenzothiophen-Index der Wadi Gaza-Sedimente bestätigt einen Eintrag von Rohöl und Erdölderivaten. Verbindungen terrestrischer Herkunft (höhere Landpflanzen), wie z.B. langkettige *n*-Alkane, *n*-Fettsäuren und *n*-Alkohole, sowie marine Verbindungen kommen ebenfalls vor.

Cyanobakterienmatten aus dem Wadi Gaza haben in den Labor- und Mesokosmos-Experimenten sowohl aliphatische (Pristan und *n*-Oktadekan) als auch aromatische Verbindungen (Phenanthren, Dibenzothiophen) effektiv abgebaut. Der Abbau der Modellverbindungen geschah in den Laborexperimenten schneller als in den Mesokosmen. Die Abbauraten sind von der Art der Verbindungen abhängig; aromatische Verbindungen werden schneller abgebaut als aliphatische Kohlenwasserstoffe.

Im Experiment mit Rohöl haben die Matten sowohl *n*-Alkane als auch die Isoprenoide Pristan und Phytan abgebaut. Am schnellsten wurden die C_{14}- bis C_{20}- *n*-Alkane abgebaut, am langsamsten die C_{21}- bis C_{27}- *n*-Alkane. Mit mittleren Raten wurden die *n*-Alkane mit 28 bis 35 Kohlenstoffatomen biodegradiert. Pristan und Phytan wurden innerhalb von 60 Tagen zu 94 und 88% abgebaut.

Die Matten zeigen eine hohe Fähigkeit, Herbizide abzubauen. 2,4-Dichlorophenoxyessigsäure wurde innerhalb von 30 Tagen sowohl im Licht als auch in Dunkelheit zu über 84% entfernt. Terbutryn wurde bis auf 28% (Licht) und 36% (Dunkelheit) der Ausgangskonzentration innerhalb von 80 Tagen abgebaut. Die beobachteten Halbwertszeiten von 49 und 56 Tagen zeigen, dass das Terbutryn schnell abgebaut wurde.

Aus den Untersuchungen ist zu schließen, dass die aus einem mit Rohöl und Erdölderivaten stark verschmutzen Gebiet stammenden Cyanobakterienmatten aus dem Wadi Gaza ein hohes Bioremediationspotential aufweisen und Erdölderivate und Herbizide abbauen können.

1. Introduction

Pollution of marine environments with petroleum and petroleum products became a world wide problem on the tide of industrialization. The Middle East is the most important petroleum producing region of the world. The Red Sea and the Eastern Mediterranean Sea serve as major routes for international oil transport from Middle East to Europe. Presently, significant oil pollution problems exist along the Eastern Mediterranean coast of the Gaza Strip.

The increasing number of marine oil spills asks for effective solutions for the environment. Bioremediation techniques have shown potential for broad applications in terrestrial and freshwater environments by treating sediment contaminated with oil and other substances, as well as for coastal environments impacted by oil spills.

Coastal marine and hypersaline cyanobacterial mats are commonly found along the Red Sea and the shores of the Eastern Mediterranean Sea. Their ability to thrive when exposed to massive contamination by large quantities of spilled crude oil was demonstrated clearly along the Kuwait and Saudi Arabian coastline during the 1991 Gulf war (Sorkhoh et al., 1992).

Evidence has been presented that microbial communities dominated by phototrophic cyanobacteria can be actively involved in the degradation of petroleum and its derivatives. Observations after oil spills in the Arabian Gulf showed intensive colonization of polluted sites by cyanobacteria-dominated microbial mats which correlated with the disappearance of hydrocarbons (Hoffmann, 1996; Höpner et al., 1996). Remarkably, crude oil reaching the cyanobacterial mat-dominated sabkha area was quickly overgrown by cyanobacterial mats which effectively biodegraded the crude oil within six months. However, large parts of the crude oil accumulated in the coastal sediments and still remain a major source of environmental pollution more than 10 years after the event. Massive cyanobacterial mats were also found in highly polluted coastal waters along the Gaza Strip and in the western part of Wadi Gaza (Gaza Strip, Palestine), a stream that receives a variety of pollutants such as diesel oil and other petroleum products, solid waste, pesticides, sewage as well as agricultural and industrial discharge.

1.1 Objectives

The main objectives of this thesis work were:

i) to study the organic geochemistry of Wadi Gaza sediments and their level of pollution, particularly with petroleum and petroleum derivatives. For this purpose

surface sediments samples were collected from different sites along Wadi Gaza and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) techniques.

ii) to investigate the ability of naturally occurring cyanobacterial mats inhabiting the heavily polluted natural environment of Wadi Gaza to degrade selected model compounds (*n*-octadecane, pristane, phenanthrene and dibenzothiophene) in small-scale laboratory experiments. The selected compounds represent important groups of petroleum constituents (straight-chain alkanes, branched alkanes, aromatic hydrocarbons and organo-sulfur compounds).

iii) to elucidate the degradation potential of the cyanobacterial mats to the same petroleum compounds in large scale as a follow-up experiment based on the results of the small-scale experiments. The experiments were carried out under field conditions in an outdoor experimental pond under the natural climate in Gaza.

iv) to study the degradation kinetics in experiments with intact cyanobacterial mats and slurries.

v) to investigate the capability of Wadi Gaza cyanobacterial mats to degrade Egyptian crude oil and 2,4-dichlorophenoxyacetic acid and terbutryn herbicides in laboratory experiments.

1.2 Organic pollutants in sediments

Pollutants are often major components of the land-borne material introduced in coastal areas and have significant impact on coastal ecosystems and public health. Research in coastal environments has thus been recognized to be critical for achieving a sustainable industrial, agricultural and communal management and ecosystem preservation.

Hydrocarbons are ubiquitous constituents in aquatic sediments. They enter the marine environment by both aquatic and atmospheric pathways, the latter consisting of dry and wet deposition. The relative importance of the two main pathways for a given environment depends on the geographical setting (Prahl et al., 1984; Gagosian and Peltzer, 1986; Lipiatuo and Albaiges, 1994).

Natural (i.e. biogenic) aliphatic hydrocarbons in sediments derive from a variety of allochthonous sources, notably higher plants, and autochthonous sources, including plankton and bacteria. Characteristic molecular compositions often provide a means for distinguishing between sources (Brassell et al., 1978; Cranwell, 1982). Biogenic hydrocarbons are typically characterized by homologous series of short-chain *n*-alkanes (e.g., *n*-C_{15}-C_{19}) if derived from

algae or longer-chain *n*-alkanes (n-C_{25}-C_{35}) if derived from higher plant epicuticular waxes, coupled with a strong predominance of odd-over-even carbon numbers.

Aliphatic hydrocarbons are also major components of petroleum products, producing a distribution in which odd and even carbon chain lengths are roughly equal in abundance, yielding a carbon preference index (CPI) of ≈ 1 and often with an unresolved complex mixture (UCM) of branched and cyclic alkanes.

Among the hydrocarbons, the polycyclic aromatic hydrocarbons (PAHs) are widespread ubiquitous environmental pollutants (e.g. Blumer, 1976; Suess, 1976; Harvey, 1996). Thus, PAHs are an important class of compounds that are prevalent in sediments in modern estuaries. PAHs have received special attention since they have long been recognized as hazardous environmental chemicals (NAS, 1975). PAHs are classified as priority pollutants by the United States Environmental Protection Agency (Keith and Telliard, 1979; Smith et al., 1989). Due to their mutagenic and carcinogenic effects on aquatic organisms, the source and fate of PAHs have become a matter of ecotoxicological interest (Christensen and Zhang, 1993; Neff, 1995; Yuan et al., 2000).

PAHs are derived from natural and anthropogenic sources. Natural sources include forest and prairie fires (Blumer and Youngblood, 1975; Venkatesan and Dahl, 1989; Killops and Massoud, 1992; Yunker and Macdonald, 2003), natural petroleum seeps and post-depositional transformations of biogenic precursors over relatively short periods of time (Wakeham et al., 1980). They can be derived from biogenic precursors like terpenes, pigments and steroids (Laflamme and Hites, 1979). Anthropogenic sources of PAHs include combustion of fossil fuel (Hites et al., 1977; Fernández et al., 2000; Yunker et al., 2002; Yunker and Macdonald, 2003), long-range atmospheric transport of PAHs adsorbed onto soot or airborn particulate matter (Lunde and Bjorseth, 1977; Laflamme and Hites, 1978), urban runoff containing PAHs derived from abrasion of street asphalt, automobile tires and vehicular emissions (Wakeham et al., 1980). PAHs are also introduced into the environment through contamination by spillage of petroleum and its refined products which contain complex assemblages of PAHs (Lake et al., 1979; Sporstol et al., 1983; Merrill and Wade, 1985; Boehm et al., 1991; Mazeas and Budzinski, 2001).

PAHs are detected in air (Koeber et al., 1999; Lim et al., 1999), soil and sediment (Huntley et al., 1993; van Brummelen et al., 1996; Zeng and Vista, 1997; Langworthy et al., 1998; Lamoureux and Brownawell, 1999; Ohkouchi et al., 1999), surface water, groundwater and road runoff (Pitt et al., 1995; Boxall and Maltby, 1997; Martens et al., 1997; Holman et

al., 1999). They are dispersed from atmosphere to vegetation (Wagrowski and Hites, 1997) and contaminate food (Lee and Grant, 1981; Edwards, 1983; Sims and Overcash, 1983).

Several studies have described the cycling of PAHs in marine environments (Broman et al., 1991; Lipiatou and Saliot 1991; Naf et al., 1992; Bouloubassi and Saliot, 1994; Wakeham, 1996). The source and physicochemical properties of the individual PAHs determine their biogeochemical fate in estuarine systems. Differential water solubilities (Eganhouse and Calder, 1976), preferential biodegradation of low-molecular-weight oil-derived PAHs with respect to higher-molecular-weight pyrogenic PAHs (Jones et al., 1986) and different particle associations of PAHs depending on their sources (Prahl and Carpenter, 1983; Readman et al., 1984; Bouloubassi and Saliot, 1993a,b) control the speciation of PAHs in marine systems.

The distributions of PAHs in sediments give information on the sources (Gschwend and Hites, 1981; Colombo et al., 1989). Combustion-derived PAHs are dominated by the unsubstituted parent compounds, whereas petroleum PAHs are dominated by the alkylated homologues (Laflamme and Hites, 1978). Substituted phenanthrenes with methyl substituents in positions 2, 3, 9 and/or 10 have been reported to occur in sedimentary material including oil shales (Radke et al., 1982b; Radke and Welte, 1983) and crude oil (Lekveishvili et al., 1980; Radke et al., 1990, 1993; Budzinski et al., 1995). The ratios of methylphenanthrenes to phenanthrene (MPhen/Phen) were examined as an indication of anthropogenic influence. These ratios were reported to be in the range of 2-6 in sediments dominated by fossil-fuel phenanthrenes (Prahl and Carpenter, 1983).

Organic sulfur compounds such as dibenzothiophene and its derivatives have been widely used as model compounds in petroleum studies (Kilbane and Bielaga, 1990). In petroleum-contaminated environments, alkyl dibenzothiophenes have been shown to be quite persistent (Hostettler and Kvenvolden, 1994; Wang et al., 1994), and they concentrate in the tissues of aquatic species (Ogata and Fujisawa, 1985). Several studies demonstrated that four methyldibenzothiophene isomers occur in sediment (Domine et al., 1994; Jiang et al., 1994; Safi et al., 2001).

1.3 Microbial Mats

Microbial mats can be found all over the world in a wide range of environments. This includes fresh water lakes and streams, hypersaline lakes and lagoons, alkaline lakes, intertidal coastal sediments, marine salterns, coral reefs, hot springs, dry and hot desert and Antarctic lakes (D'Amelio et al., 1989; Pierson 1992; Stahl, 1995). They represent the

world's smallest ecosystem (Karsten and Kühl, 1996) sharing very close morphological similarities to their alleged fossil Precambrian analogue, i.e. they are modern analogues of 3.5 billion year old stromatolites, laminated carbonate build-ups that preserve evidence of the earth's earliest biosphere (Stanley, 1984; Reid et al., 2000).

Microbial mats are cohesive, accretionary biofilms growing on solid surfaces with typical macroscopically observable vertical laminations which arise from the different pigments of the distinct physiological groups of microorganisms in the mats (Stal and Caumette, 1994). Most locations where microbial mats develop are characterized by extreme conditions of salinity, temperature, moisture or pH. Such conditions are responsible for the absence of efficient grazers and seem to be a prerequisite for mat expansion (Farmer, 1992; Castenholz, 1994). Microbial mats vary in thickness and can accumulate to a thickness of more than one meter (Stal and Caumette, 1994). They can cover areas of several square kilometers (Des Marais et al., 1992). There are categorical differences between modern microbial mats, e.g. marine/saline mats and hot spring mats (Castenholz, 1994), but most of them are dominated by a few functional groups of microorganisms (van Gemerden, 1993).

Cyanobacterial mats are composed of physiologically different groups of microorganisms such as cyanobacteria and diatoms as oxygenic phototrophs, chemolithotrophic colorless sulfur bacteria as aerobic heterotrophs, anaerobic phototrophic purple sulfur bacteria (anoxygenic phototrophs) and anaerobic sulfate-reducing bacteria (Figure 1.1). Other numerically less important groups in mats are nitrifying/denitrifying bacteria and methanogenic bacteria. Due to the spatial separation (lamination) of diverse

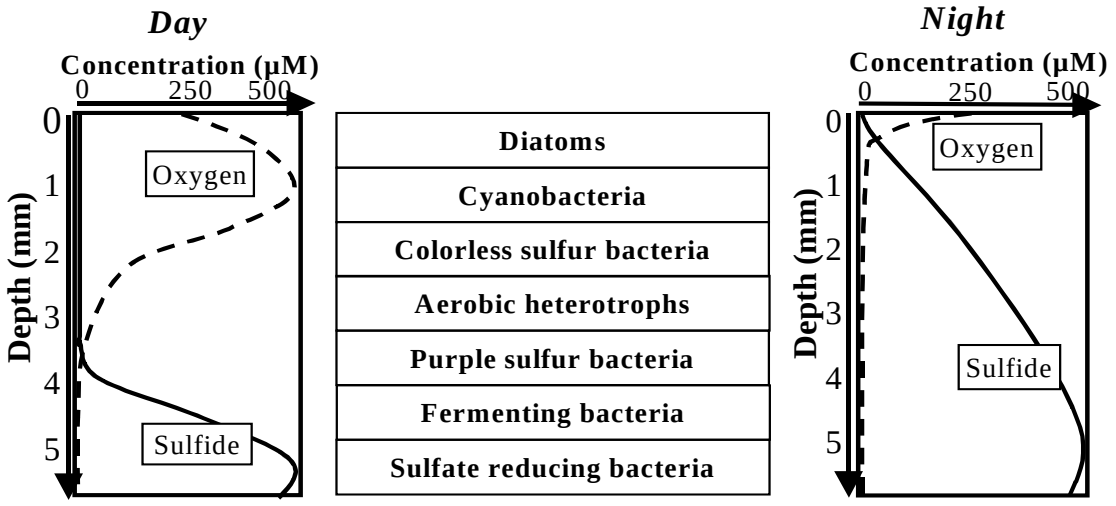


Figure 1.1. Schematic composition of a microbial mat in which the different functional groups of microorganisms appear to be vertically layered. Oxygen and sulfide concentrations fluctuate due to the bacterial activity in response to changing phototrophic conditions (van Gemerden, 1993, modified by Jonkers, 1999).

metabolisms of mat-inhabiting groups of microorganisms, steep physico-chemical gradients of light, oxygen and sulfide are developed (Revsbech et al., 1983; Des Marais 1995; Stal 1995).

Filamentous cyanobacteria play an important role in the colonization of new sites. Krumbein (1994) reported that filamentous cyanobacteria stabilize the sediment by trapping sand grains in excreted exopolymers. Representatives of this group can be found in a large number of microbial mats, often dominating the upper few millimeters (Jørgensen et al., 1983; Stal, 1995). Oxygenic phototrophs like diatoms (algae) can also occur in the upper layer of microbial mats, but eukaryotic organisms are often excluded from extreme environments where mats are found because of their limited range of metabolic capabilities as compared to prokaryotes (Stal, 2000). By contrast, the prokaryotic cyanobacteria not only perform oxygenic photosynthesis, but are also capable of photoheterotrophy, sulfide-dependent anoxygenic photosynthesis and fermentation. Their wide range of metabolic capacities enable them to survive changing environmental conditions. The production of oxygen and organic carbon by photosynthetic activity is an important function of cyanobacteria in microbial mats. Their photosynthetic activity drives the rest of the microbial community.

The organic matter produced by excretion, decomposition and lysis of cyanobacteria is respired by aerobic heterotrophs, leading to oxygen depletion and regeneration of CO_2, which is then used by autotrophic organisms. The same organic compounds, as well as low-molecular-weight compounds produced by fermentative bacteria, can also be used by sulfate-reducing bacteria under anoxic conditions producing hydrogen sulfide. The sulfide is inhibitory to cyanobacteria but can be re-oxidized to sulfate by both colorless and purple sulfur bacteria. During the daytime the photosynthetically active surface layer of the mat is supersaturated with oxygen whereas CO_2 concentration is low. In contrast, during the night, anoxic conditions tend to prevail in mats and, if sulfate is available, sulfide accumulates.

Fermentation of intracellular storage compounds, like glycogen, by cyanobacteria can result in the excretion of ethanol, propionate, acetate and lactate (Nold and Ward, 1996). Diurnal variations of light intensity bring about the formation of steep opposing gradients of oxygen and sulfide, which move up and down in the microbial mat. Hence the mats naturally contain physiologically flexible microorganisms tolerant to these fluctuating conditions. For example, some cyanobacterial species have higher rates of oxygenic photosynthesis in the presence of sulfide, a substrate that is toxic to most cyanobacteria, whereas others carry out sulfide-dependent anoxygenic photosynthesis (Cohen et al., 1986; Stahl, 1995). On the other

hand, some sulfate-reducing bacteria, traditionally regarded to be strictly anaerobic organisms, may occur in the upper oxic layer of the mats (Teske et al., 1998).

Microbial mats are a promising ecosystem for biodegradation studies because of their high internal turnover rates of carbon compounds, changing diurnally, their steep physicochemical gradients and the presence of many different physiological groups of microorganisms. Several reports have demonstrated the ability of microorganisms belonging to such physiological groups to biodegrade petroleum compounds.

1.4 The environmental situation of Wadi Gaza

Geologically, the Wadi Gaza area is part of a coastal plain, which consists of a series of geological formations dipping gradually from east to west. These geological formations are mainly of Tertiary and Quaternary age.

The Wadi Gaza region was subject to a downward movement during the Miocene, which included an intrusion of sea water toward the east, the formation of a sea gulf and the deposition of a 500 m thick sequence of limestone, sandstone and chalk. Then another sequence of 1000 m thickness of clay and loam mixed with seashells formed during the Pliocene at the end of the Tertiary. This layer is impermeable to water. Also, a clay layer next to the bottom of the Wadi was formed at the beginning of the Quaternary as a result of the flooding of the Wadi (Moshtaha, 1999).

Wadi Gaza is one of the largest streams in Palestine. It runs in east-west direction across the Gaza Strip. The drainage basin of Wadi Gaza covers more than 3500 km^2 of the Northern Negev Desert and the Hebron Mountains as well as the small catchment in Gaza. The Wadi‘s length from its origin to its mouth is about 105 km, of which the last 9 km are in the Gaza Strip (Figure 1.2). Wadi Gaza has two main tributaries. One is Wadi Alshari‘a which collects water from the Hebron Mountains in the West Bank and the other one is Wadi Alshallala which collects water from the Northern Negev heights. Wadi Gaza continues to flow as one stream through the Gaza Strip down to the Mediterranean Sea with a slope of about 1:450, which is almost flat, whereas its slope in the upper parts in the Negev and Hebron Mountains is about 1:100 (Awadallah, 2000).

The width and depth of Wadi Gaza vary a lot on the way to the Mediterranean Sea. The width ranges between 40 and 60 m in the middle and eastern part, whereas in the west at its mouth to the sea it becomes more than 400 m wide. The depth of the wadi varies from 6 to 12 m in the east and flattens to 3-4 m in the west till it reaches zero at the mouth.

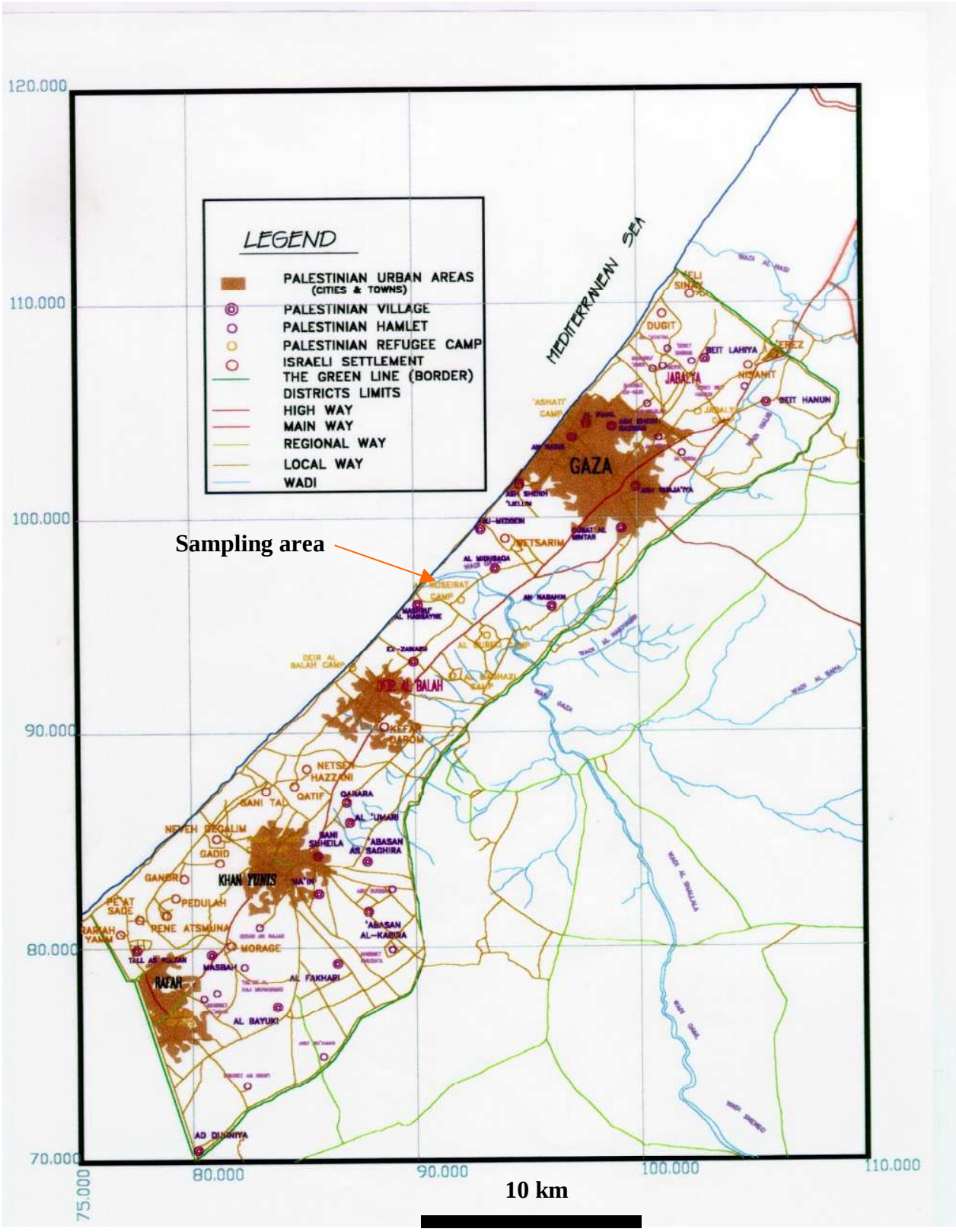


Figure 1.2. Map of Gaza Strip showing Wadi Gaza and the sampling area in the western part of the Wadi (Palestinian Water Authority).

The vegetation in the wadi is dominated by tamarisks growing on the dunes and sand deposits in and around the wadi bed. The wetter areas have stands of Typha which also fringe the water body near the outlet to the sea. However, around 125 ha of salt marshes recorded in the Gaza Environmental Profile of 1994 have disappeared following construction of the bridge at the mouth of Wadi Gaza in 1996.

Wadi Gaza as well as the whole Gaza Strip area is located in the transitional zone between the temperate Mediterranean climate to the west and north and the arid desert climate of the Negev and Sinai deserts to the east and south. The Wadi Gaza area has a characteristical semi-arid climate. The average daily mean temperature ranges from 25°C in summer to 13°C in winter, with an average daily maximum temperature range from 29°C to 17°C and a minimum temperature range from 21°C to 9°C in summer and winter, respectively. The annual average rainfall is 335 mm, and the mean daily evaporation is about 2.1 and 6.3 mm in December and July, respectively.

Coastal and seawater pollution is one of several serious problems currently affecting the Gaza Strip (Safi, 1995). Wadi Gaza is heavily used for disposal of sewage and solid waste as well as agricultural and industrial wastewater. The eastern part of the wadi is influenced by untreated wastewater discharge. The effluent from the Gaza City treatment plant is discharged into Wadi Gaza and then disappears in the dunes and partly reaches the sea (Nashashibi and van Duijl, 1995). In the west, the connection to the Mediterranean Sea is intermittent depending on rainfall; a sand bar separates the wadi from the sea at times of low water level. Most of the cyanobacterial mats develop in the western part of the wadi in the presence of a high level of pollution by diesel oil and other petroleum products (Figure 1.3) (Safi et al., 2001). They are naturally exposed to fluctuating salinity due to seasonal seawater intrusion and freshwater run-off. Temperature, salinity, and water level of the wadi change seasonally, leading to marked changes in the appearance of the mats.

The Wadi gains in its importance from the fact that it is the only surface water resource in the Gaza Strip as well as being the main natural feature of Gaza which makes it a major place for biodiversity. The Wadi Gaza area is a site of significant importance to migrating birds from Europe to Africa in autumn and from Africa to Europe in spring.

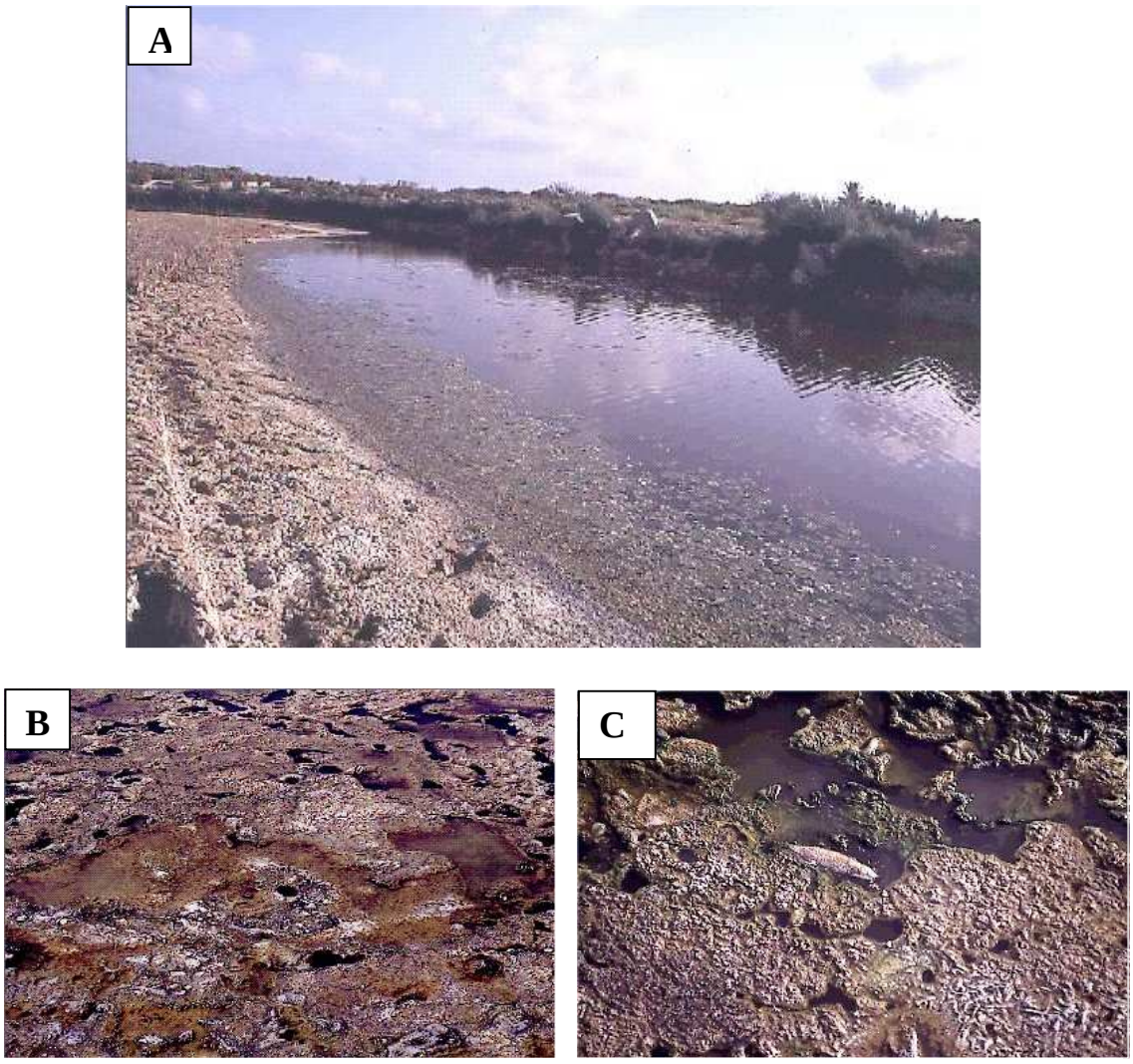


Figure 1.3 A: The heavily polluted site of Wadi Gaza located at the Mediterranean coast shows a remarkable development of cyanobacterial mats. B and C: Sediment polluted with petroleum derivatives

1.5 Composition of crude oil

Crude oil is found in large quantities below the surface of the earth and can be used as fossil fuel or raw material in the chemical industry. It is often refined into kerosene, gasoline, diesel fuel and other products.

Crude oil is a complex mixture of thousands of compounds, but each accumulation of oil tends to be unique in composition (Radwan and Al-Hasan, 2000, and references therein). In terms of element composition, crude oil is composed predominantly of carbon and hydrogen with an H/C ratio of about 1.85 (Hunt, 1979). Other elements such as sulfur, nitrogen and oxygen are present in minor proportions (less than 3%). Traces of phosphorous and heavy metals such as vanadium and nickel are also usually present (Hunt, 1996; Radwan et al., 1999).

Petroleum compounds range in molecular size from very small molecules such as methane to large ones like asphaltenes with molecular weights in the tens of thousands. Crude oils, irrespective of their origin, can be split into a number of fundamental chemical classes on the basis of their structure, e.g. saturated hydrocarbons, aromatic hydrocarbons, resins and asphaltenes (Figure 1.4). For more details see Colwell and Walker (1977), Hunt (1979) and Tissot and Welte (1984).

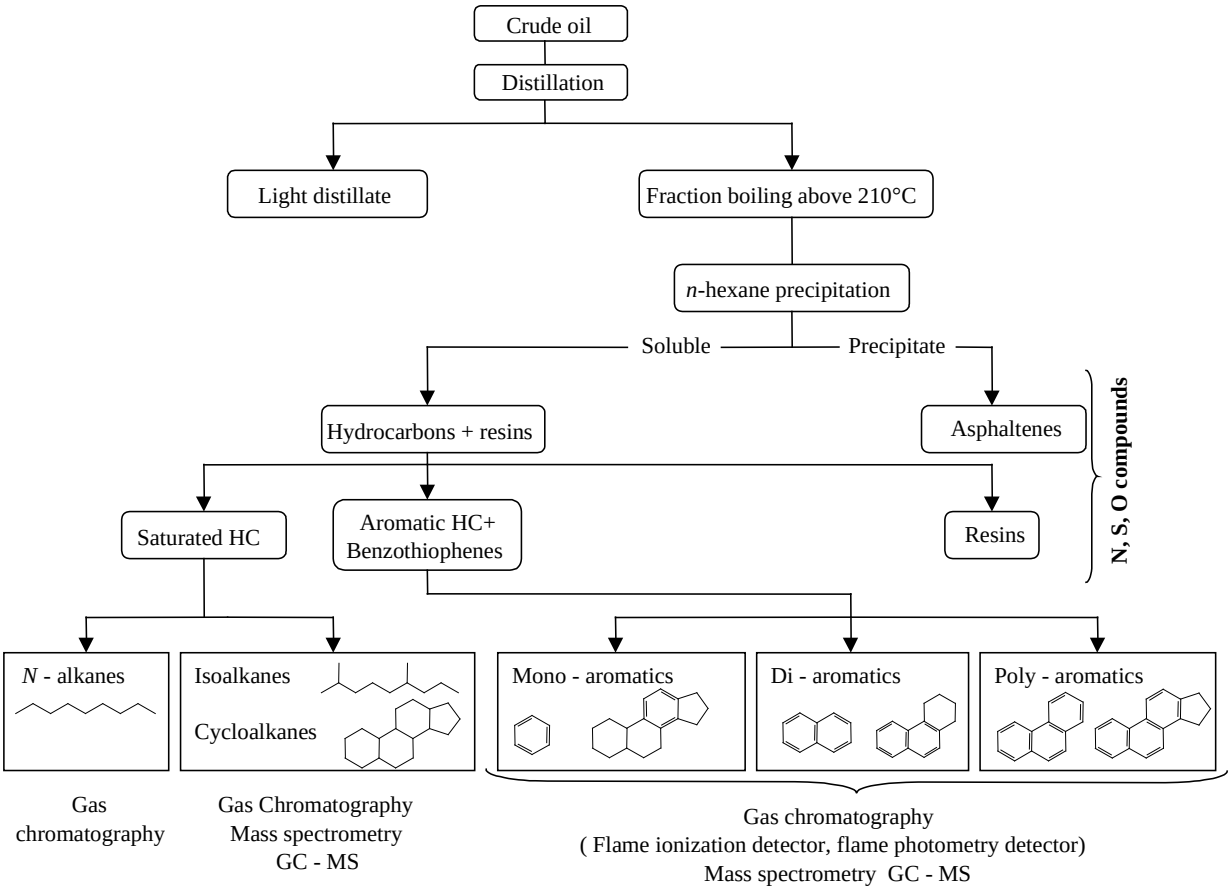


Figure 1.4. Flow chart showing crude oil composition, separation of main structural types of molecules and subsequent quantitative analysis of fractions by gas chromatography and mass spectrometry (modified after Tissot and Welte, 1984).

1.6 Oil spills: Impact on the environment

The earth has faced many disasters which were caused by humans throughout history. Today one of the most important hazards jeopardizing marine environments are marine oil spills. The marine environment is subject to contamination by petroleum and other hydrocarbons from a variety of sources. Contamination results from uncontrolled effluents from

manufacturing and refining installations, spillages during transportation, direct discharge from treatment plants and run-off from terrestrial sources.

In quantitative terms, crude oil is one of the most important organic pollutants in marine environments. It has been estimated that worldwide somewhere between 1.7 and $8.8x10^6$ tons of petroleum hydrocarbons impact marine waters and estuaries annually (NAS, 1985). Other researchers give even higher figures. For example, data summarized by Konovalov (1995) suggest that global oil release into the World Ocean reaches 20 million tons a year, and pollution caused by tankers accounts for 50% of it (http://www.offshore-environment.com/oilpollution.html). The number of oil tanker accidents is increasing with the amount of oil transported on the seas. In 1988, $1.050\ x10^{12}$ tons of crude oil were on the road (Frees, 1992).

Several large accidental oil spills resulted in significant contamination of marine and shoreline environments (Swannell et al., 1996). For example, observations in the Caribbean basin, where annually up to 1 million tons of oil enter the marine environment, showed that about 50% of this amount came from tankers and other ships. In the Bay of Bengal and the Arabian Sea, oil pollution from tankers and other ships equals $4x10^5$ and $5x10^6$ tons of oil a year, respectively (http://www.offshore-environment.com/oilpollution.html). Off Spain about $5x10^5$ tons of oil were released from the oil tanker Prestige in November 2002 and about 240 km of Spain's beaches were affected by the spill. During the 1991 Gulf War the release of about one million tons of crude oil into the Arabian Gulf led to the largest oil spill in human history (Purvis, 1999). The oil spill erased most of the intertidal plant and animal communities along 770 km of coastline from southern Kuwait to Abu Ali Island in Saudi Arabia (Krupp et al., 1996). The Exxon Valdez oil spillage of $4x10^4$ tons into Prince William Sound, Alaska, created the largest spill ever with respect to the more than 2,000 km of oiled shoreline. A substantial number of smaller releases of petroleum hydrocarbons occur regularly in coastal waters. Around the coast of the UK alone, in the years of 1986-1996 6845 oil spills were reported. Of these, 1497 occurred in environmentally sensitive areas or were of sufficient magnitude to require clean-up.

Once the oil is spilled it is subject to physical, chemical and biological changes. Abiological weathering processes include evaporation, dissolution, dispersion, photochemical oxidation, water-in-oil emulsification, adsorption onto suspended particulate material, sinking and sedimentation. In oil spills, the volatile hydrocarbon fractions evaporate quickly, leaving behind longer-chain aliphatic and aromatic components (Madigan et al., 2000).

These compounds are destructive to various forms of marine life. Pollution with oil may also lead to general killing of fish, shellfish, birds and other invertebrates (Atlas and Bartha, 1998). The Exxon Valdez disaster killed more wildlife than any other environmental disaster, including an estimated 3500-5500 sea otters, 300 harbor seals and 14-22 killer whales. Seabird expert Dr. Michael Fry said the Exxon Valdez spill killed nearly ten times as many birds as any other U.S. or European oil spill. As many as half a million birds died, including bald eagles, harlequin ducks, marbled murrelets and loons (http://jomiller.com/exxonvaldez/report.html).

The presence of such a large amount of oil spread over a large area poses serious environmental hazards to air, land and ground water as well as to humans (Saeed et al., 1998). It is particularly important to address oil-polluted waters as soon as possible, as the contamination can have the potential to damage fishery resources and affect the health of those animals and humans that consume contaminated fish (Krahn and Stein, 1998).

As a consequence of the importance of oil spills relative to other sources of organic contaminants in the marine environment, there is a large body of research on oil-spill bioremediation. Furthermore, studies of oiled shorelines have been far more numerous than open water studies, which have often been equivocal (Swannell et al., 1996; Prince, 1997).

1.7 Biodegradation and bioremediation

Environmental damage due to oil spills in the past and recent times has focused on the need for environment-friendly strategies for remediation of the contaminated sites. Based on the fact that biodegradation was the major process for removal of non-volatile oil components from the environment, bioremediation has been projected to play an important role in environmental cleanup (Swanell and Head, 1994).

Bioremediation is not a new concept. Microorganisms have been breaking down chemicals in their environment to use them as a food source since the first microbe evolved on our planet over four billion years ago (Brock and Madigan, 1991). Microbiologists have studied the process since the 1940s. However, bioremediation became known to a broader public only as a technology for cleanup of shorelines contaminated with spilled oil. The Amoco Cadiz oil spill in Brittany in 1978 and the Exxon Valdez oil spill in 1989 in Prince William Sound, Alaska, were the catalysts for this attention. In the years since 1989, bioremediation has become a technology that is discussed, applied and considered in many different circumstances (Hoff, 1993).

Biodegradation is the partial breakdown or complete destruction of the molecular structure of environmental pollutants by physiological reactions catalyzed by microorganisms (Atlas and Cerniglia, 1995; Madsen, 1997; Alexander, 1999). It is routinely measured by applying chemical and physiological assays to laboratory incubations of flasks containing pure cultures of microorganisms, mixed cultures or environmental samples (sediment, water or soil) (Madsen, 1998). *Bioremediation* is the intentional use of biodegradation processes to eliminate environmental pollutants from sites where they have been released either intentionally or inadvertently. Bioremediation technologies use the physiological potential of microorganisms, as documented most readily in laboratory assays, to eliminate or reduce the concentration of environmental pollutants in field sites (NRC, 1993; Shauver, 1993). Microbial processes may destroy environmental contaminants *in situ*, where they are found in the landscape, or *ex situ*, which requires that contaminants be mobilized out of the landscape into some type of containment vessel (a bioreactor) for treatment.

Intrinsic bioremediation is passive, it relies on the innate capacity of microorganisms present in the field sites to respond to and metabolize the contaminants. Because intrinsic bioremediation occurs in the landscape where both indigenous microorganisms and contaminants reside, this type of bioremediation necessarily occurs *in situ*. For intrinsic bioremediation to be effective, the rate of contaminant destruction must be faster than the rate of contaminant migration. These relative rates depend on the type of contaminant, the microbial community and the site's hydrogeochemical conditions (Madsen, 1998). Intrinsic bioremediation has been documented for a variety of contaminants and habitats (Madsen et al., 1991; Murarka et al., 1992; Wilson and Madsen, 1996), e.g. gasoline-related compounds in groundwater (Salanitro, 1993), crude oil in marine waters (Bragg et al., 1994), and low-molecular-weight chlorinated solvents in groundwater (Major et al., 1991; Semprini, 1995).

Engineered bioremediation takes an active role in modifying a site to encourage and enhance the biodegradative capabilities of microorganisms. Selection of the most effective bioremediation strategy is based on characteristics of the contaminants (toxicity, molecular structure, solubility, volatility and susceptibility to microbial attack), the contaminated site (geology, hydrology, soil type, climate, the legal and economic situation) and the microbial process that will be exploited, such as pure culture, mixed cultures, and their respective growth conditions, and supplements (Tiedje, 1993; Madsen, 1997). Bioremediation and its recent development as an oil spill response technology provide an interesting example of how a new environmental technology comes into being.

1.8 Organo-clay complexes

The increasing use of organic compounds and petroleum products poses a serious impact to human health and the environment. Such environmental problems must be controlled in order to minimize the harmful effects of these products. One problem frequently encountered with these compounds is deterioration of marine life and contamination of groundwater (Koterba et al., 1993; Thurman et al., 1996; Weber et al., 1999). It is highly desirable to develop methods that would render these organic compounds in soils or water available for biodegradation and/or mineralization and, thus, reduce the contamination of ground waters and marine life.

Clay is an important fraction in soil. It determines the fate of many organic chemicals, which may directly or indirectly reach the soil. The inorganic exchangeable ions of natural clays, i.e. Na and Ca, are strongly hydrated in the presence of water, resulting in a hydrophilic environment at the surface of the clay. As a result, natural clays are ineffective sorbents for poorly water-soluble, non-ionic organic compounds (NOCs), such as the aromatic hydrocarbons that frequently move from contaminated sites (agricultural and/or industrial areas) into the subsurface and the groundwater.

In case of hydrophilic clays, adsorption of non-ionic organic compounds is suppressed in the presence of water because relatively non-polar organic chemicals cannot effectively compete with the highly polar water for adsorption sites on the clay surface. In the presence of water, the clay acts as a conventional sorbent; the high adsorptive capacity for organic compounds is attributed to its large surface area (Chiou and Shoup, 1985). The type of exchangeable cations on clay minerals strongly influences their sorptive characteristics for NOCs.

The inability of clays to adsorb substantial amounts of NOCs from aqueous solution can be altered by replacing natural metal cations with larger organic cations through ion-exchange reactions (McBride et al., 1977; Wolfe et al., 1985; Boyd et al., 1988). Earlier studies indicate that exchanging quaternary ammonium cations for metal ions on clays greatly modifies the sorptive characteristics of dry clays for organic vapours (McAtee and Harris, 1977). The influence of surfaces on microbial activities and the ways in which specific attributes of bacteria affect their ability to utilize sorbed substrates are long-standing questions in microbial physiological ecology (Marshall, 1976; Fletcher, 1985; van Loosdrecht et al., 1990).

The influence of sorption on the biodegradation of organic contaminants has been recognized as an important, albeit poorly understood issue in bioremediation (Guerin and

Boyd, 1992). The chemical structure of the compound sorbed, the nature of the sorbent, the residence time of the sorbed compound and the desorption rate may influence the degradation of sorbed compounds (Crocker et al., 1995). Fractions of 2,4-dichlorophenoxy acetic acid (2,4-D; Ogram et al., 1985; Greer and Shelton, 1992), polyaromatic hydrocarbons (PAHs; Weissenfels et al., 1992), naphthalene (Mihelcic and Luthy, 1991) and phenol (Scow and Alexander 1992) sorbed to clay were available for biodegradation. The bioavailability of sorbed compounds may also be affected by the microorganisms themselves. Guerin and Boyd (1992, 1997) used a kinetic method to show that the ability to directly utilize soil-sorbed naphthalene is a species-specific characteristic. *Pseudomonas putida* strain 17484 was able to directly access labile sorbed naphthalene and promote the desorption of the non-labile compounds from the interior of soil particles. In contrast to strain 17484, *Alcaligenes* sp. Strain NP-ALK utilized only naphthalene from the aqueous phase, and most of the soil-sorbed fraction remained unavailable.

The main idea behind the use of organo-clay complexes is to create a hydrophobic environment on the clay surfaces. This environment would enhance the migration of the organic pollutants from the aqueous phase to the solid phase and accumulate them on the surface. This accumulation may enhance the bio-availability of these compounds for the bacteria. Thus, a continuous degradation process may occur. Creation of such a hydrophobic environment on the clay surfaces would happen by simple ionic exchange reactions using suitable organic cations. This modification may better bind the non-ionic organic pollutants in the water and, thus, maximize the remediation process of the cyanobacterial mats.

In the present study benzyl trimethyl ammonium clay (clay-BTMA) was used because it has larger a adsorption capacity than other complexes for aromatic compounds (El-Nahhal et al., 1998, 1999) and a higher stability under saline solutions (El-Nahhal and Safi, 2004).

Petroleum compounds are considered to be recalcitrant to microbial degradation and persist in ecosystems because of their hydrophobic nature (low water solubility) and low volatility, and thus they pose a significant threat to the environment. To overcome the low accessibility of these compounds in the degradation experiments, organo-clay complexes were used: the model compounds were adsorbed to modified clay particles with hydrophobic surfaces in order to enhance the contact of the pollutants with the microbial community and to increase their bioavailability.

1.9 Degradation of crude oil

Petroleum hydrocarbons are widespread environmental pollutants. Crude oil is one of the most important organic pollutants in the marine environment and one of the most dangerous pollutants in the biosphere. The annual oil loss, with the current volume of oil production, is about 50 million tons (Orlov et al., 1991). The biodegradation of water-insoluble organic compounds of high molecular weight such as heavy oils, asphaltenes or industrial crude oil residues has been the subject of great interest for many years.

Bioremediation is gaining increased acceptance as a strategy for the treatment of marine oil systems impacted by petroleum hydrocarbon release (Head and Swannell, 1999). Generally, the more the crude oil is refined, the easier it is for the micro-organisms to degrade it. Paraffinic constituents of crude oil differ in their susceptibility to biodegradation: *n*-Alkanes > alkycyclohexanes > acyclic > isoprenoid alkanes such as pristane and phytane > bicyclic alkanes > steranes > hopanes > diasteranes (Volkman et al., 1983). Degradation of shorter-chain alkanes is more readily achieved than that of longer-chain alkanes (van Beilen et al., 1994; Whyte et al., 1998). The degradation pathways of *n*-alkanes are described in the following section.

There is increasing interest in cyanobacterial mats as tools for self-cleaning and bioremediation of polluted coastal environments (Kuritz and Wolk, 1995). Cyanobacterial mats are known to be important hydrocarbon degraders in the sea (Leahy and Colwell, 1990). The first recognition of a possible role for microbial mats in bioremediation was made after the Persian Gulf war 1990-91. The intertidal zones of the Kuwaiti and Saudi coast, about 700 km along the Gulf, became heavily polluted with crude oil. The pioneer colonizers of this oily environment were cyanobacteria that grew as thick mats on the top of oily sediments (Figure 1.5) (Sorkhoh et al., 1992; Al-Hasan et al., 1994). The final areal extent of cyanobacterial mat development was 1.55 km^2, and the oil spill did not damage the cyanobacterial mat sites but rather promoted their extensive development to a high degree, with a good possibility of survival even in habitats where they did not occur before, such as *Cleistostoma* crab colonies (Barth, 2003). Cyanobacterial mats, which commonly develop in extreme environments including polluted ones

Figure 1.5. Cyanobacterial mats colonizing heavily contaminated sediment in the Arabian Gulf (Höpner, 1993).

(Gibson and Smith 1982; Fogg, 1987; Sorkhoh et al., 1992), are the most important primary producers in the Persian Gulf (Golubic, 1992; Sheppard et al., 1992; Al Thukair and Al Hinai, 1993). Cyanobacterial mats dominated by *Microcoleus chthonoplastes* and *Lyngbya aestuarii* grow on weathered oil residues covered with sediment (Hoffmann, 1994, 1996).

Degradation of oil in the marine environment is reported to occur with mixed populations of native microbes (Atlas 1991, 1995; Harayama et al., 1996) and in microbial mats (Al-Hasan et al., 1994). Oil-degrading microorganisms are also effective in laboratory experiments (e.g., Petrikevich et al., 2003). In the Arabian Gulf, mats were directly involved in the disappearance of the oil by both biodegradation and physical removal (Höpner et al., 1996). The organotrophic bacteria *Acinetobacter calcoaceticus* and *Micrococcus* sp. isolated from oily sediments of the Arabian Gulf degraded petroleum compounds (Radwan et al., 1999). Mixed cultures of the marine cyanobacterial species *Oscillatoria salina*, *Plectonema terebrans* and *Aphanocapsa sp*. degraded Bombay High crude oil and *Aphanocapsa sp*. degraded pure *n*-hexadecane (Raghukumar et al., 2001). *Acinetobacter* sp. A3 isolated from crude oil refinery sludge was able to degrade Bombay High crude oil and utilize it as the sole source of carbon (Hanson et al., 1996, 1997). *Klebsiella* sp. KCL-1 isolated from seawater used *n*-alkanes of crude oil, from *n*-octadecane to *n*-hexacosane, as a sole carbon source (Cha et al., 1999). Most *n*-alkanes in Kuwait crude oil were degraded by *Pseudomonas* strains (Al-Gounaim et al., 1995).

A strain of *Yarrowia lipolytica* isolated from a chronically oil-polluted tropical marine estuary degraded most of the aliphatic fraction of Bombay High crude oil (Zinjarde et al., 2000). *Rhodococcus sp*. isolated from a chronically oil-polluted marine site partially degraded the aliphatic fraction of crude oil (Milekhina et al., 1998; Whyte et al., 1998; Sharma and Pant, 2000). *n*-Alkanes of an Egyptian crude oil were degraded by a mixed culture of bacteria (El-Rafie et al., 2001) and by mixed microbial populations collected from different sites of oil spills (Amin et al., 1995). In aerobic biodegradation of crude oil carried out in the laboratory, Pond et al. (2001) found that C_{14} to C_{28} *n*-alkanes were degraded. The most rapidly degraded homologues were in the range from C_{14} to C_{18}. Medium- and long-chain *n*-alkanes with up to C_{40} carbon atoms served as sole carbon and energy source for *Acinetobacter calcoaceticus* at the Arabian Gulf coast polluted with oil (Radwan et al., 1999). Normal and branched alkanes of Alwyn crude oil were biodegraded by the halotolerant bacterial consortium MPD-M (Díaz et al., 2002). The biodegradation rates for *n*-alkanes in crude oil tend to decrease as chain length increases (Holder et al., 1999; Pond et al., 2001). Natural microbial populations in seawater also biodegraded crude oil (Dutta et al.,

2000). Palittapongarnpim et al. (1998) reported bacterial degradation of 40% of *n*-alkanes of Tapis crude oil within seven days.

1.10 Degradation of petroleum model compounds

Wadi Gaza cyanobacterial mats have been shown to degrade petroleum model compounds in slurry experiments in the laboratory (Safi et al., 2001; Abed et al., 2002) and in outdoor experiments with intact mats (Safi et al., 2003) as will be shown in more detail in the results section. Degradation of both aliphatic and aromatic hydrocarbons has been reported for hypersaline microbial mats (Grötzschel et al., 2002).

Other studies have demonstrated the capacity of cyanobacterial isolates to degrade hydrocarbons (Cerniglia et al., 1980a,b; Cerniglia, 1992; Al-Hasan et al., 1998; Raghukumar et al., 2001). Two non-axenic cultures of *Microcoleus chthonoplastes* and *Phomidium corium*, isolated from oil-rich sediments of the Arabian Gulf, are responsible for degrading *n*-alkanes (Al-Hasan et al., 1998). *Oscillatoria* sp. JCM was able to oxidize biphenyl to 4-hydroxybiphenyl (Cerniglia et al., 1980c) and naphthalene to 1-naphthol (Narro et al., 1992a). *Agmenellum quadruplicatum* PR-6 converted phenanthrene into phenanthrene-*trans*-9,10-dihydrodiol (Narro et al., 1992b). Degradation of a mixture of 11 different PAHs, including phenanthrene, by using enrichment cultures from a soil contaminated with diesel fuel has been reported (Eriksson et al., 2003). Thermophilic bacteria obtained from crude oil were able to degrade 98% of dibenzothiophene within 15 days (Bahrami et al., 2001). A *Bacillus* species degraded phenanthrene and utilized naphthalene, biphenyl and anthracene as growth substances (Doddamani and Ninnekar, 2000). Strain *Sphyingomonas* sp. 2MPII, isolated from a marine sediment, was able to utilize phenanthrene as the sole carbon source (Nadalig et al., 2002).

The biodegradation of *n*-alkanes occurs by monoterminal, diterminal and subterminal oxidation pathways. Organisms of the *Rhodococcus* group have been reported to possess enzymes for either the monoterminal (Warhurst and Fewson, 1994), diterminal (Broadway et al., 1993) or only the subterminal oxidation pathway (Ludwig et al., 1995). Occurrence of both terminal and subterminal modes of catabolism of alkanes have also been reported (Woods and Murrell, 1989; Whyte et al., 1998). In the monoterminal pathway the primary alcohol is formed followed by the aldehyde and the monocarboxylic acid (Foster, 1962a,b; Linden and Thijsse, 1965; McKenna and Kallio, 1965; Vaneyk and Bartels, 1968; Ratledge, 1978). Further degradation of the carboxylic acid proceeds by β-oxidation with subsequent formation of two-carbon-unit shorter fatty acids and acetyl coenzyme A, with eventual

liberation of CO_2. Fatty acids have been found to accumulate during hydrocarbon biodegradation (Atlas and Bartha, 1973; King and Perry, 1975). An omega (diterminal) oxidation pathway has also been reported (Jurtshuk and Cardini, 1971; Watkinson and Morgan, 1990). A subterminal oxidation pathway sometimes occurs, with formation of the secondary alcohol and subsequently the ketone, but this does not appear to be the primary metabolic pathway followed by most *n*-alkane-utilizing microorganisms (Markovetz, 1971). Whyte et al. (1998) reported both subterminal and monoterminal oxidation of *n*-C_{12} and *n*-C_{16} alkanes by *Rhodococcus* sp. strain Q15 producing 1-dodecanol and 2-dodecanone, and 1-hexadecanol and 2-hexadecanol, respectively.

Dioxygenase systems also exist in some microorganisms. The *n*-alkanes are initially oxidized to the corresponding hydroperoxide and then transformed to the primary alcohol (Watkinson and Morgan, 1990) or to the aldehyde, as originally postulated by Finnerty (1977) and demonstrated by Sakai et al. (1996) in *Acinetobacter* sp. strain M-1. Monocarboxylic and dicarboxylic acids were produced by a marine *Rhodococcus* sp. when grown on odd and even carbon number alkanes, respectively (Sharma and Pant, 2000).

Pristane oxidation has been reported in the *Rhodococcus* group of organisms. Production of both monoic and dioic acids of pristane by *Rhodococcus* sp. BPM 1613 has been reported by Nakajima et al. (1985). Prinik et al. (1974) reported similar results for *Brevibacterium erythrogens*. Cells grown on pristane produced only 2,6,10,14-tetramethylpentadecanoic acid in the monoterminal oxidation pathway during pristane conversion by *Rhodococcus* sp. NCIM 5126 (Sharma and Pant, 2000).

The pathways of phenanthrene metabolism by bacteria have been reported by several investigators (Kiyohara et al., 1976; Kiyohara and Nagao, 1978; Kiyohara et al., 1982; Barnsely, 1983; Ghosh and Mishra, 1983; Gibson and Subramenian, 1984). The initial reaction is the action of dioxygenase followed by oxidation to form 3,4-dihydroxyphenanthrene, which subsequently undergoes *meta*-cleavage and is converted to 1-hydroxy-2-naphthoic acid. 1-Hydroxy-2-naphthoic acid can be further degraded via two routes. In one route it undergoes ring cleavage to form *o*-phthalic acid and protocatechuic acid, which is finally cleaved to form pyruvic acid and ultimately enters the tricarboxylic acid (TCA) cycle (Kiyohara et al., 1976; Kiyohara and Nagao, 1978; Ghosh and Mishra, 1983; Houghton and Shanley, 1994). In the other route, 1-hydroxy-2-naphthoic acid undergoes oxidative decarboxylation to form 1,2-dihydroxynaphthalene, which is then subject to *meta*-cleavage to form salicylic acid (Gibson and Subramenian, 1984). Salicylic acid can also be further degraded via the formation of either catechol or gentisic acid. Both

catechol and gentisic acid undergo ring fission to form TCA-cycle intermediates (Houghton and Shanley, 1994).

Dibenzothiophene has been widely used as model organic sulfur compound in petroleum studies (Kilbane and Bielaga, 1990). Three pathways of dibenzothiophene degradation have been reported. The first is the ring-destructive pathway, in which the sulfur of dibenzothiophene remains (Kodama et al., 1970; Laborde and Gibson, 1977; Monticello et al., 1985; Crawford and Gupta, 1990). The second is the completely destructive pathway, in which dibenzothiophene is mineralized to carbon dioxide, sulfite and water (van Afferden et al., 1990), and the third is the sulfur-specific pathway, in which only sulfur is removed from dibenzothiophene (Kilbane and Jackowski, 1992; Omori et al., 1992; Olson et al., 1993).

In the sulfur-specific pathway, dibenzothiophene is oxidized to the sulfoxide, then to the sulfone (Krawiec, 1990; Gallagher et al., 1993; Shennen, 1996). Subsequently, the sulfur ring is cleaved, leaving 2-hydroxybiphenyl and releasing sulfite, which is spontaneously oxidized to sulfate. Bacteria such as *Gordona* sp. strain CYKSI (Rhee et al., 1998), *Rhodococcus erythropolis* strain D-1 (Izumi et al., 1994; Matsubara et al., 2001), *Corynebacterium* sp. (later reclassified as *Rhodococcus*; Omori et al., 1995), strain SY-1 (Omori et al., 1992) and *Rhodococcus* sp. strain IGTS8 (Kilbane and Jackowski, 1992; Matsubara et al., 2001) are capable of this mode of attack. They use dibenzothiophene as their sole sulfur source. Dibenzothiophene sulfone is converted to 2-hydroxybiphenyl 2-sulfinic acid. The latter one can be desulfurized to 2-hydroxybiphenyl by *Rhodococcus erythropolis* strain D-1 (Matsubara et al., 2001). Kodama et al. (1973) identified 4-(3-hydroxy-2-benzo[b]thienyl-2-oxo-butenoic acid) as a metabolite from dibenzothiophene. Three products were identified as 3-hydroxy-2-formyl-benzothiophene, benzothienopyran-2-one and dibenzothiophene-5-oxide from the degradation of dibenzothiophene by *Rhizobium meliloti* Orange 1 isolated from oxic sediments polluted by oil refinery leakage (Frassinetti et al., 1998). 3-Hydroxy-2-formyl-benzothiophene has been reported as a metabolite of dibenzothiophene degradation by *Pseudomonas* strains 1-MN and BT1d (Kropp et al., 1997).

1.11 Degradation of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and terbutryn

The Gaza Strip is suffering from catastrophic environmental conditions. The uncontrolled and heavy use of pesticides is one of several serious problems currently affecting the Gaza Strip (Safi, 1995, 2002). More than 350 metric tons of formulated pesticides (insecticides,

fungicides, nematicides, herbicides and others) are used annually in Gaza Strip (Safi, 2002). 2,4-Dichlorophenoxyacetic acid (2,4-D) and terbutryn are commonly used herbicides in the Gaza Strip. In 1998, about 1 ton of 2,4-D and 0.8 ton of terbutryn and ametryne were used in the Gaza Strip.

Increased public awareness of environmental pollution has brought to the forefront the need for new technologies to help mitigate deterioration of environmental quality. Contaminated sites are often bioaugmented with bacteria with specific properties or capabilities, such as contaminant degradation.

1.11.1 Degradation of 2,4-dichlorophenoxyacetic acid (2,4-D)

The synthetic herbicide 2,4-D ($C_8H_6Cl_2O_3$), a chlorinated phenoxy compound, has been used extensively both domestically and commercially for almost 50 years (Loos, 1975). It rapidly became the most widely used herbicide in the world and remains so to date (Rhoades, 2001). It is a selective herbicide with preferential activity against dicotyledons and is used against broadleaf plants in agriculture, forest management, homes, in pasture and rangeland applications and to control aquatic vegetation. 2,4-D is used in different formulations as a salt-based water-miscible solution, as an ester-based emulsifiable concentrate or as solid alkali salt concentrate. The water solubility ranges from 0.3 g/l at pH 1 to 34.2 g/l at pH 9 at 25°C (WHO, 1978; ARS, 1995; Tomlin, 2000). It is used in mixtures with other herbicides and represents a main constituent of the defoliant Agent Orange (Zhao et al., 1987).

2,4-D is slightly to moderately toxic to mammals and humans, but highly toxic in aquatic environments (Stevens and Sumner, 1991; Anonymous, 1994; Tomlin, 2000; NLM, 2001). At 2,4-D concentrations between 1 and 10 µM, photosynthesis in aquatic microorganisms is inhibited; 10 µM can inhibit growth by as much as 50%, depending on the species and environmental conditions (Koch, 1989). When exposed to 2.2 mg/l 2,4-D, the algae *Chlorella pyrenoidosa*, *Lyngbya* sp., *Chlorococcum* sp. and the cyanobacterium *Anabaena variabilis* were unaffected in terms of respiration, photosynthesis and growth (Hawxby et al., 1977). At a 2,4-D concentration of 0.01 g/l the growth and heterocyst formation of the cyanobacterium *Nostoc* was reduced, and growth stopped at concentrations above 1 g/l (Gangawane et al., 1980).

The relatively short half-life time of 2,4-D in soil previously exposed to the herbicide is largely attributed to the activity of bacteria, representing several different genera, with the ability to cometabolize or completely mineralize the herbicide (Loos, 1975; Sinton et al.,

1986). There are many studies available on the degradation of 2,4-D by microorganisms, and it is an excellent model compound for the study of degradation of chlorinated aromatic compounds in the environment. A number of 2,4-D degraders have been isolated (Loos et al., 1979; Fournier, 1980; Don and Pemberton, 1981; Ou,1984; Ka et al., 1994a; Fulthorpe et al., 1995; Maltseva et al., 1996; Park and Ka, 2003). Most of the characterized strains belong to the β and γ subdivisions of the class *Proteobacteria* which contain 2,4-D-degrading genes similar to the *tfd* genes found in the strain *Alcaligenes eutrophus* JMP134 (Don and Pemberton, 1981; Kaphammer et al., 1990; Kaphammer and Olsen, 1990; Fukumori and Hausinger, 1993; Matrubutham and Harker, 1994; Kamagata et al., 1997). Other isolates were composed of strains in the α subdivision of *Proteobacteria*, to date mostly in the genus *Sphingomonas* (Ka et al., 1994a,b,c).

The wide use of 2,4-dichlorophenoxyacetic acid has prompted interest in its biodegradation. Microbial degradation of 2,4-dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid used as sole carbon and energy substrates has been reported (Sandmann et al., 1988; Smith and Lafond, 1990). 2,4-Dichlorophenoxyacetic acid, in particular, has been widely studied because it serves as a model compound for understanding the mechanism of biodegradation of other, structurally related haloaromatic compounds. Genera of bacteria reported to degrade 2,4-dichlorophenoxyacetic acid include *Pseudomonas* (Kilpi et al., 1980), *Alcaligenes* (Pieper et al., 1988), *Arthrobacter* (Beadle and Smith, 1982) and *Flavobacterium* (Chaudhry and Huang, 1988).

The degradation pathways of 2,4-D have been characterized by Chaudhry and Huang, (1988) and Sandmann et al. (1988). Chlorophenols are metabolites of 2,4-dichlorophenoxy acetic acid and also degradation products of numerous chlorinated organic compounds (Evans, 1963; Reinecke and Knackmuss, 1988). 2,4-Dichlorophenol has been reported as a metabolite of 2,4-D in soils (Smith and Aubin, 1991a). It is subject to a rapid dissipation in soil by formation of nonextractable residues (Smith and Aubin, 1991b). 2,4-Dichlorophenol can be rapidly mineralized in soil (Boyd et al., 1989). Plasmid $_{p}$JP4 from *Alcaligenes eutrophus* JMP134 converted 2,4-D to chloromaleylacetate (Top et al., 1995). Further metabolism of chloromaleylacetate to tricarboxylic acid cycle intermediates occurrs by enzymes encoded by chromosomal genes of JMP134 (Don et al., 1985; Kukor et al., 1989).

Cyanobacteria were shown to degrade both naturally occurring aromatic hydrocarbons (Ellis, 1977; Cerniglia et al., 1979; Cerniglia et al., 1980a,b; Narro, 1992b) and xenobiotics (Megharaj, 1987). Fifteen strains of cyanobacteria that belong to three taxonomic groups are able to degrade the highly toxic chlorinated pesticide lindane (lambda-

hexachlorocyclohexane; Kuritz, 1999). This metabolic feature was first reported for the two filamentous cyanobacteria *Nostoc ellipsosporum* and *Anabaena* sp. (Kuritz and Wolk, 1995). Most of 21 species of freshwater algae isolated from a natural lake fully degraded 2,4-D to butoxyethanol ester within two weeks, and seven isolates reduced 2,4-D to less than 20% of the amount added (Butler et al., 1975). Six species of microorganisms isolated from soil previously treated with herbicides completely degraded 2,4-D after 20 to 30 days (Le, 1984). Pure cultures of *Pseudomonas aeruginosa* and *Pseudomonas species* or mixed cultures from a 2,4-D manufacturing plant effluent grew on 2,4-D as the sole source of carbon and energy (Buenrostro-Zagal et al., 2000; Musarrat et al., 2000). By a combination of anaerobic and aerobic steps, many chlorinated compounds were completely degraded (Fathepure and Vogel, 1991; Gerritse and Gottschal, 1992; Gerritse et al., 1997). Since in microbial mats periods of oxygen oversaturation with anoxia change within 24 h, such mats represent a promising system for the biodegradation of halogen-substituted hydrocarbons.

1.11.2 Degradation of terbutryn

Terbutryn ($C_{10}H_{19}N_5S$; N-(1,1-dimethylethyl)-N′-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine) is a triazine which is a selective herbicide. It has been tested for use as an aquatic herbicide in several countries over the past 35 years (after Murphy et al., 1980). Triazine herbicides are common contaminants of surface water of agricultural regions (Morris and Jarman, 1981). Terbutryn is absorbed by the root and foliage and acts as an inhibitor of photosynthesis. It is a pre-emergent and post-emergent control agent against most grasses and many annual broadleaf weeds in winter wheat, winter barley, sunflowers, sugar cane, sorghum and, in mixture with terbuthylazine, on peas, beans and potatoes. It is also used as a directed spray in maize (U.S. Department of Health and Human Services, 1993; Tomlin, 2000) and as an aquatic herbicide for control of submerged and free-floating weeds and algae in water courses, reservoirs, and fish ponds (Unwin Brothers Ltd, 1994). Terbutryn has been determined in surface and groundwaters (Carabias-Martinez et al., 2003). It is used in different formulations as a wettable powder or a soluble concentrate (Meister, 1992). The water solubility is 22 mg/l at 22°C (Tomlin, 2000).

Terbutryn is slightly toxic to birds (U.S. EPA, 1986; Unwin Brothers Ltd, 1994; Tomlin, 2000) and moderately toxic to fish (Meister, 1992; Tomlin, 2000). It affects the central nervous system in animals leading to convulsions, incoordination or labored breathing. At extremely high dosages, the animals showed swelling and fluid in the lungs and the central nervous system (Wagner, 1981). The enzyme activities of rainbow trout

decreased when exposed to terbutryn (Tarja et al., 2003). Terbutryn is not a skin sensitizer, and no mutagenic effects were observed. It has been classified as a possible human carcinogen by the U.S. EPA (1986). Toxic effects on aquatic microorganisms have been emphasized. Much of this research has dealt with phototrophic algae and cyanobacteria, which are the main primary producers in the hydrosphere (El-Dib et al., 1989). The growth of a freshwater microalga (*Chlorella vulgaris*) was strongly inhibited after exposure to terbutryn (Rioboo et al., 2002). At terbutryn concentrations between 1.0 and 4.0 mg/l, the algal growth was reduced to 56%, and the ratio of Chl(a)/Chl(b)/Chl(c) of Nile algae was affected. After exposure to terbutryn, *Oscillatoria formosa*, *Oscillaton'a mougeotii* and *Lyngbya limnetica* were the most dominant species (Badr and Abou-Waly, 1997).

Triazines are amongst the most widely used herbicides. Since triazines can be found in many environmental compartments, their fate in ecosystems and the characterization of their degradation in the environment need to be determined. Terbutryn is readily adsorbed in soils with high organic or clay content (Meister, 1992). There are very few published studies on the degradation of terbutryn in the environment. Soil microorganisms play an important role in the degradation of terbutryn. The half life in soil is 14-28 days (Unwin Brothers Ltd, 1994). Depending on the application rate, the residual activity of terbutryn in soil is 3 to 10 weeks (Tomlin, 2000). It is slightly mobile to immobile in soil. Its major breakdown product, hydroxyterbutryn, is more mobile and persistent and has potential to leach to the groundwater (Meister, 1992).

In water, terbutryn is not volatile, and it is adsorbed by suspended particulate matter and sediment. Degradation of terbutryn in aquatic systems is caused by microbial processes. (Tomlin, 2000). Half-lives of 180 and 240 days have been reported for the degradation of terbutryn in river sediment and pond, respectively (Muir and Yarechewski, 1982). Terbutryn and it's degradation products, N-deethylated terbutryn, hydroxyterbutryn and N-deethylated hydroxyterbutryn were found in water, sediment and aquatic plants (Muir, 1980).

2. Materials and Methods

2.1 Microbial mat and sediment samples

2.1.1 Environmental setting of the microbial mats

The mat samples used in this study were from Wadi Gaza, Gaza Strip, Palestine. This wadi originates at Hebron in the West Bank and flows in east-west direction across the Gaza Strip. It is the only surface water in the Gaza Strip and is heavily used for disposal of sewage, solid waste, and agricultural and industrial wastewater. In the west, Wadi Gaza reaches the Mediterranean Sea. The connection to the sea is intermittent, depending on rainfall. At the sampling sites most of the cyanobacterial mats develop in the presence of high levels of pollutants, mainly diesel oil and other petroleum products (Safi et al., 2001). The salinity, temperature and water level of the Wadi change seasonally, which results in marked variations in the appearance of the mats. In the western part of the wadi, the salinity ranges from 1% (wt/vol) total salts in the winter to 3.6% (wt/vol) total salts in the summer. The average daily temperature varies between 13°C in the winter and 35°C in the summer.

2.1.2 Collection and preservation of mat samples

Cyanobacterial mat samples (10 cm x 15 cm and 12 cm x 20 cm) were collected at different sites along the Wadi Gaza in May 2000 and August 2001, respectively. At the time of sampling, the mats were submerged, the measured water temperature was 25°C and 31°C and the salinity 2% and 3.6%, respectively. The mats were transferred to the laboratory at the Institute of Chemistry and Biology of the Marine Environment (ICBM) in Oldenburg (Germany) within 24 hours. Upon arrival, the mats collected in May 2000 were incubated in a glass aquarium (32 x 32 x 15 cm, l/w/h) (Figure 2.1).

Figure 2.1. Cyanobacterial mats collected from Wadi Gaza in May 2000.

Mats collected in August 2001 were incubated in another glass aquarium (40 x 25 x 25 cm, l/w/h) which at the bottom contained a layer of sterilized sea sand of up to 3 cm thickness (Figure 2.2). The two aquaria were filled with seawater from the North Sea (Germany) to a level of 10 cm and 20 cm height, respectively. The salinity was adjusted to 2% (wt/vol), the temperature ranged between 25 and 32°C and the pH was 8.1. The mats were incubated under a light regime of 12 hours light/ 12 hours dark with an intensity of ca. 80 µmol photons/m^2s. The water depth was monitored and adjusted to 10 cm and 20 cm, respectively.

Figure 2.2. Cyanobacterial mats collected from Wadi Gaza in August 2001.

2.1.3 Sediment samples

Fifteen sampling sites were selected along the Wadi Gaza; six sites in the eastern part and nine sites in the West. Sediment samples were collected from the fifteen sites in January 2000, and another four samples were collected from the western part of the Wadi in August 2001. All the sediment samples were transferred to the laboratory at the Environmental Protection and Research Institute (EPRI) in Gaza, Gaza Strip, Palestine, within 1 hour and kept in a deep freezer at –60°C over night. The samples were then transferred within 24 hours to the laboratory at ICBM in January 2000 and September 2001, respectively.

2.2 Laboratory experiments

2.2.1 Preparation of organo-clay complexes for the slurry experiments

n-Octadecane ($C_{18}H_{38}$), molecular weight 254.49 g/mol, purity 99%, pristane ($C_{19}H_{40}$), molecular weight 268.5 g/mol, purity 99%, phenanthrene ($C_{14}H_{10}$), molecular weight 178.23 g/mol, purity 98%, and dibenzothiophene ($C_{12}H_8S$), molecular weight 184.26 g/mol, purity 98%, (Sigma-Aldrich) were used as model compounds for petroleum constituents representing straight-chain alkanes, branched alkanes, aromatic hydrocarbons and aromatic

organo-sulfur compounds, respectively. The chemical structures of the organic compounds are shown in Figure 2.3.

Hydrophobic clay was used as a carrier for the petroleum model compounds in our experiments in order to enhance the contact of the compounds with the microbial community and to increase their bioavailability. A 2% aqueous suspension of montmorillonite KSF (Aldrich) was prepared, and 0.8 mmol of benzyl-trimethylammonium (BTMA) chloride per g of clay was added slowly as a 10 mM aqueous solution (procedure modified after El-Nahhal et al., 2000). The mixture was stirred for 24 h, washed three times with water to remove excess BTMA chloride and then freeze-dried.

To adsorb the model compounds, the hydrophobic clay (BTMA montmorillonite) was suspended in *n*-hexane. The mixture of model compounds (20 mg per 100 mg of hydrophobic clay, dissolved in *n*-hexane) was slowly added with continuous stirring. The slurry was treated in a vacuum rotary evaporator to remove the *n*-hexane. This yielded a homogeneous powder consisting of hydrophobic clay loaded with 16.67% (wt/wt) petroleum model compounds (designated the organo-clay complex, OCC). To verify the amount of loaded model compounds, the OCC was re-extracted with dichloromethane (DCM) and the extract analyzed by gas chromatography.

2.2.2 Slurry experiment with petroleum model compounds

Experiments were carried out in sterile 250 ml Erlenmeyer flasks. The medium used was prepared by mixing equal volumes of natural seawater and distilled water. The salinity was adjusted to 2% to mimic the salinity at the Wadi Gaza site at the time of sampling. Nitrogen and phosphate sources were added to the medium by way of 1 mM ammonium chloride and 8 µM sodium dihydrophosphate, respectively. Each Erlenmeyer flask, except the control flasks, was filled with 100 ml autoclaved medium, 1 g Wadi Gaza mat material and 100 mg organo-clay complex.

The following four controls, two for chemical analysis and two for community structure comparison, were used: (1) medium and organo-clay complex without mat material (incubated in the light and in the dark); (2) medium and organo-clay complex with autoclaved mat material; (3) medium and mat material without organo-clay complex, and (4) medium, mat material and hydrophobic clay without model compounds. The first two controls were used to check for photooxidation of the model compounds and to account for adsorption of the organo-clay complex particles to the mat material, respectively. The other two controls were used to monitor changes in the community in the absence of model

compounds. All flasks were incubated at 28°C with constant shaking at 100 rpm and a light regime consisting of 12 h light and 12 h darkness. The light intensity was 80 µmol of photons m^{-2} s^{-1} (photosynthetically available radiation). One flask containing medium, organo-clay complex particles and mat inoculum was incubated in complete darkness. The experiment was performed for 40 days, and samples used for chemical analysis (2 ml each) were taken every 4 days during the first 2 weeks and every 10 days until the end of the experiment. At the end of the experiment, the contents of the flasks were collected by centrifugation.

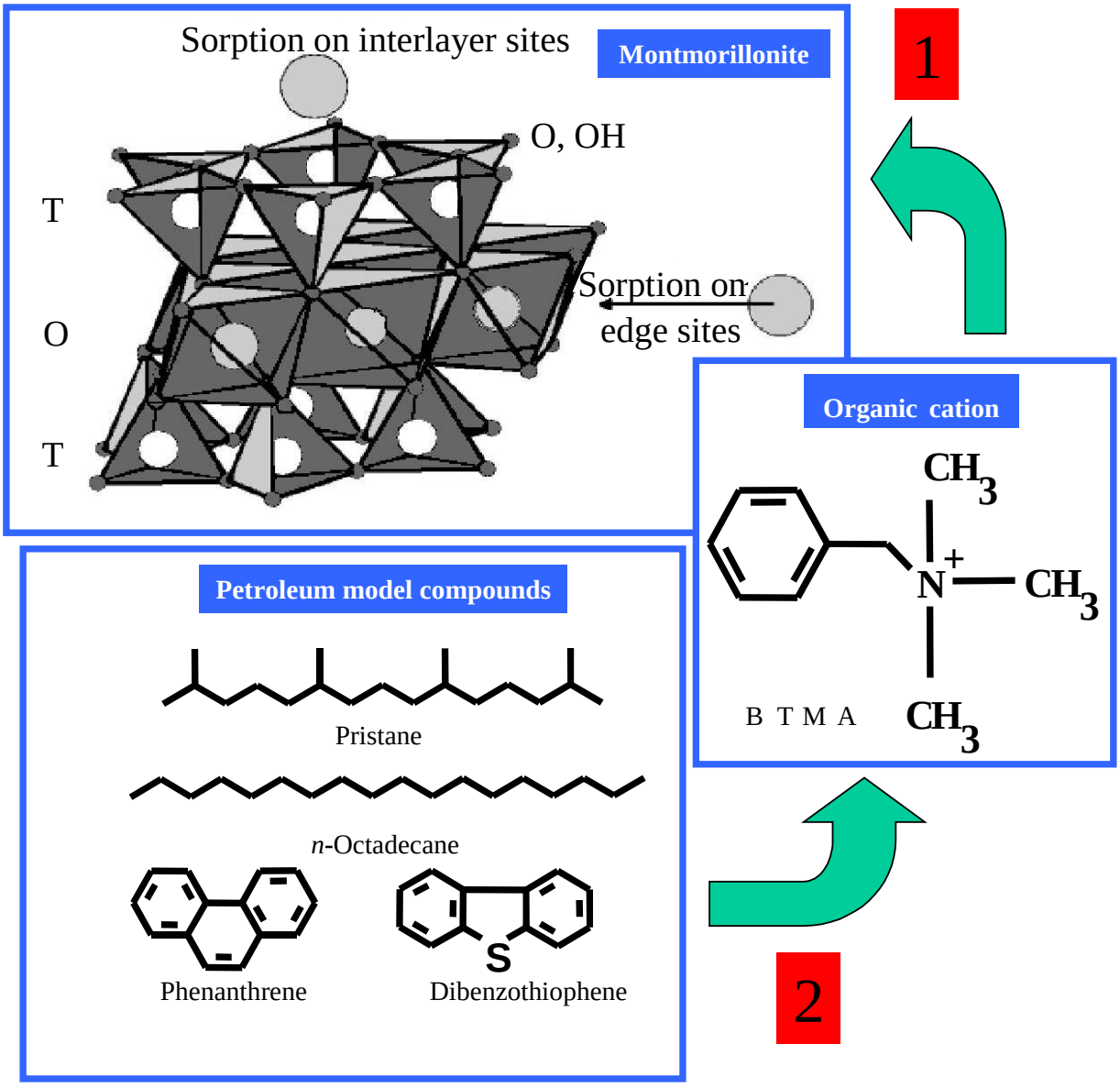


Figure 2.3. Organo-clay complex (OCC): Four petroleum model compounds, each of them representing structural types present as major constituents in petroleum, were adsorbed on organo-clay complexes. The hydrophobic organo-clay had previously been prepared by sorption of benzyl-trimethyl-ammonium cations to montmorillonite.

2.2.3 *Slurry experiment with oil-loaded organo-clay complexes*

2.2.3.1 Stabilization of the crude oil

The Egyptian crude oil (3.2 g) was transferred to a round-bottom flask, and the volatile compounds were removed by rotary evaporation (80°C, 37 mbar) for 20 min. The weight loss was 412 mg, i.e. ca. 12.8%. The oil was analyzed separately by gas chromatography before and after stabilization.

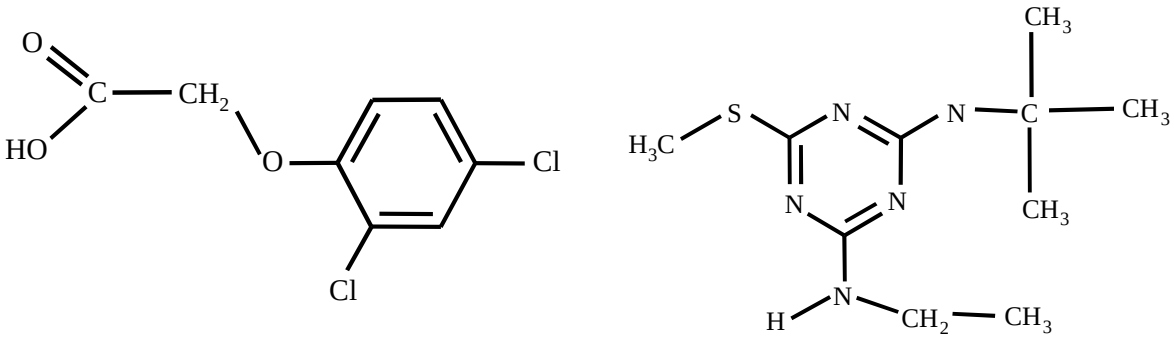
2.2.3.2 Preparation of oil-loaded organo-clay complexes

Oil-loaded organo-clay complexes were prepared essentially as described for the petroleum model compounds (2.2.1). The stabilized crude oil (1 g) was dissolved in DCM. To adsorb the crude oil, the hydrophobic clay (BTMA-montmorillonite) was suspended in DCM. The crude oil solution (1 g per 5 g hydrophobic clay, dissolved in DCM) was slowly added under continuous stirring. DCM was removed in a vacuum rotary evaporator. This yielded a homogeneous powder consisting of hydrophobic clay loaded with 16.67% (wt/wt) crude oil (designated the oil-loaded organo-clay complex) which was transferred to a small glass vial and stored in a refrigerator at 4°C prior to the degradation experiments. The autoclaved medium used for the oil degradation experiment was prepared by the same procedure as described above for the petroleum model compounds experiment. 100 ml autoclaved medium, 1 g Wadi Gaza mat material and 100 mg oil-clay complex were transferred to sterile 250 ml Erlenmeyer flasks. Control flasks received 100 ml autoclaved medium and 100 mg oil-clay complex without mat material (incubated in the light and in the dark). The light controls were used to check for photooxidation of the crude oil. The experiments were carried out for 60 days under the same conditions as described for the petroleum model compounds experiment. Samples used for chemical analysis (2 ml each) were taken with disposable pipettes at time zero and after 2, 4, 7, 10, 15, 20 days and every 10 days thereafter. At the end of the experiment, the contents of the flasks were collected by centrifugation.

2.2.4 Slurry experiment with 2,4-D and terbutryn herbicides

2.2.4.1 Chemicals

2,4-Dichlorophenoxyacetic acid (2,4-D) ($C_8H_6Cl_2O_3$), molecular weight 221.04 g/mol, purity >99%, was purchased from Merck Eurolab GmbH, Germany. Terbutryn, 2-*t*.-butylamino-4 ethylamino-6-methylthio-1,3,5-triazine ($C_{10}H_{19}N_5S$), molecular weight 241.36 g/mol, purity = 98.7%, ammonium chloride (NH_4Cl), purity >99%, and di-sodium hydrogen phosphate ($Na_2HPO_4.2\ H_2O$), purity >99.5%, were purchased from Sigma-Aldrich Chemicals GmbH, Germany. The chemical structures of the herbicides are shown in Figure 2.4.



2,4-Dichlorophenoxyacetic acid (2,4-D) Terbutryn

Figure 2.4. Chemical structures of the used herbicides.

2.2.4.2 Terbutryn experiment

The medium used was prepared by mixing equal volumes of natural seawater and distilled water. The salinity was adjusted to 2% to mimic the salinity of the Wadi Gaza site at the time of mat sampling. Nitrogen and phosphate sources were added to the medium by way of 1 mM ammonium chloride and 8 µM sodium dihydrophosphate, respectively. Terbutryn 3µg/ml was dissolved in the autoclaved medium.

The experiment was carried out in sterile 250 ml Erlenmeyer flasks. Each Erlenmeyer flask, except the control flasks, received 120 ml autoclaved medium and 1 g Wadi Gaza mat material. The control contained dissolved terbutryn in autoclaved medium without mat material. The control was used to check for photooxidation of the terbutryn. All flasks were incubated at 28°C with constant shaking at 100 rpm and a light regime consisting of 12 h light and 12 h darkness. The light intensity was 80 µmol of photons m^{-2} s^{-1} (photosynthetically available radiation).

The experiment was performed for 60 days, and samples used for chemical analysis (5 ml each) were taken every 4 days during the first 2 weeks and every 10 days until the end of the experiment. At the end of the experiment, the contents of the flasks were collected by centrifugation.

2.2.4.3 2,4-D Experiment

For the 2,4-D experiment the autoclaved medium used was prepared by the same procedure as described above for the terbutryn experiment. 14 mg 2,4-D were dissolved in 700 ml autoclaved medium at 25°C (final concentration = 20 µg/ml). The pH was 8.4. The experiment was carried out in sterile 250 ml Erlenmeyer flasks. Each Erlenmeyer flask, except the control flasks, received 100 ml autoclaved medium containing dissolved 2,4-D and 1 g Wadi Gaza mat material from the aquarium maintained since May 2000. The same procedure was followed with the Wadi Gaza mat material from the aquarium maintained since September 2001 (Figure 2. 5). The four controls used contained dissolved 2,4-D in autoclaved medium without mat material. The controls were used to check for photodegradation of the 2,4-D. Half of the flasks were incubated at temperatures ranging between 27 and 32°C with constant shaking at 100 rpm and a light regime consisting of 12 h light and 12 h darkness. The light intensity was ca. 80 µmol of photons m^{-2} s^{-1} (photosynthetically available radiation). The other half was incubated at 28°C with constant shaking at 100 rpm and 24 h darkness. The experiment was performed for 30 days. For chemical analysis of 2,4-D, 2 ml samples of the solution were taken at time zero and after 5, 10, 12, 17, 22, 27 and 30 days. At the end of the experiment, the contents of the flasks were collected by centrifugation.

Figure 2.5. Illustration of the 2,4-D slurry experiment.

2.2.5 Microscopy and cultivation

Small pieces of microbial mats from Wadi Gaza were separated and mounted in water on glass microscopic slides. The slides were examined by transmitted light microscopy, phase-

contrast microscopy, and fluorescence microscopy. Different cyanobacterial morphotypes were identified and photographed.

2.3 Mesocosm experiments

2.3.1 Experimental ponds at Gaza

Two cylindrical shaped concrete ponds (height 60 cm above the ground, effective internal diameter 6 m, total net area 27.5 m^2) were constructed in the year 2000 at the experimental station of EPRI to allow mesocosm experiments to be performed under partly controlled conditions (Figure 2.6a). The site is close to the coast of the Mediterranean Sea, and has limited vegetation and human activity.

One pond acted as a reference pond. It was used as a reservoir for freshly developed uncontaminated microbial mats for biodegradation experiments and served as a control for the development of untreated mats (Figure 2.6a). The second pond was divided into six sections (Figure 2.6b), each of which can act as a separate section for contamination experiments. Both ponds were covered on the inside with white thermoplastic sheets (FRII) purchased from Erez Thermoplastic Products to prevent any contact or chemical reactions between the water of the ponds and the concrete.

2.3.2 Collection and pre-inoculation of microbial mats for pond experiments

Sea sand collected from the sea shore was sieved through 2 mm sieves. A layer of 3-4 cm of the sieved sea sand was placed on the bottoms of the reference and sectioned ponds. Seawater was added to both ponds to a level that slightly exceeded the sand layer at the bottom. Samples of natural cyanobacterial mats (30 x 25 cm) were collected at different locations along the Wadi Gaza in October 2000. The mats were transferred within one hour to the laboratory at EPRI and well mixed to give homogeneous microbial mat material. The homogeneous suspension was transferred to the pond station and distributed over the surface of the reference pond in order to have a complete coverage of the surface.

2.3.3 Pond operation and monitoring

After distributing the mat material in the reference pond, a new microbial mat developed. Then seawater was added to a level not exceeding 33 cm. The seawater of the reference pond

was replaced on September 10, 2001. Monitoring of the ponds for water level, salinity, pH, and temperature were carried out by EPRI personnel. Chemical analyses for nitrate, phosphate, sulfate and hardness of the water in the reference pond and in the sections of pond two, of Wadi Gaza water and of seawater were also performed at EPRI.

The salinity of the pond was measured in three depth layers (0-10 cm, 10-20 cm and 20-30 cm, the surface layer). Three water samples were collected from each layer, and the salinity was measured using an optical refractometer. Standard salinity solutions, of 0.0, 10, 20, and 30 g/l were prepared to assure good quality results. The salinity was recorded three times for each layer from different locations in the pond. Averages of the measurements were calculated.

The pH values from different water depths were measured using a Micro-Temp pH 500 portable pH-meter after calibration using buffer solutions with pH 4 and 7. Three readings were recorded for each depth layer.

The temperature of each water layer was recorded using a mercury thermometer (29.8 cm) scaled to 200^{o}C. In this procedure the thermometer was immersed in the water layer manually and held for 3 minutes to insure exact reading. Then the thermometer was rapidly pulled up and reading was recorded immediately.

A scaled stainless steel ruler with a length of 50 cm adjusted to 0 cm from the bottom was used to monitor the water depth. In case of water evaporation, chlorine free water was used to recharge the pond. This water was collected from a nearby well used for agricultural irrigation.

2.3.4 Microbiological monitoring of microbial mats

Microscopic appearance, identification and distribution of cyanobacterial morphotypes within the mat samples were monitored on a weekly basis before and during the contamination experiments. Samples were collected from the reference pond, control section and contaminated sections in three separate glass vials. An aliquot from each glass vial was spread over a glass microscopic slide, covered and then examined with an Olympus microscope (BX40) with a mounted camera at EPRI. Transmitted light microscopy, phase-contrast microscopy and fluorescence microscopy were performed at the Max Planck Institute for Marine Microbiology (MPI) in Bremen (Germany).

Figure 2.6a. Reference pond at EPRI (Gaza) inoculated with microbial mats from Wadi Gaza. It is used as a reservoir for freshly developed uncontaminated microbial mats for biodegradation experiments.

Figure 2.6b. Section pond at EPRI (Gaza). It is divided into six sections which were used for controls and contamination experiments with petroleum model compounds.

2.3.5 Contamination experiment with intact mats in the sectioned pond (petroleum model compounds)

2.3.5.1 Inoculation of the sectioned pond

The bottom of the sectioned pond was covered with sieved sea sand up to 3 cm height to act as a medium for cyanobacterial mats growth and to prevent any possible contact between the mats and the thermoplastic sheets. Up to 30 cm of seawater were transferred to the sectioned pond. Cyanobacterial mats were transferred from the reference pond as pieces (30 x 25 cm) in July 2001 and arranged next to each other to have a complete coverage of the bottom of the section. Sea sand for the control section was sterilized in a hot air oven at 105°C oven for 6 h. After cooling, the sand was transferred to a glass aquarium in one of the pond sections.

2.3.5.2 Preparation and application of the organoclay-model compounds for the mesocosm experiments

Material

The clay used was sodium montmorillonite SWy-1 (Mont) obtained from the Source Clays Repository, Clay Minerals Society, Columbia, MO. Its cation exchange capacity (CEC) was 0.8 mmol/g clay (Rytwo et al., 1991). Preparation of the organically modified clay and loading with model compounds was performed as described in section 2.2.1.

2.3.6 Summer experiment

The summer experiment was carried out from 22nd July till 30th October, 2001 under field conditions in the outdoor sectioned pond (area 4.5 m^2) under the natural climate in Gaza. The medium used (1350 liter) was natural seawater. The salinity range was between 4 and 6% depending on evaporation. Water depth, temperature, salinity and pH values in the sectioned and reference ponds were monitored three times a week for the purpose of compensating evaporation by fresh water maintaining a water column of about 30 cm heigh and avoiding extreme salinity excursions.

Organo-clay complex (90 g) with 20% model compounds corresponding to 50 µg/mg of each compound was suspended in 2 liter seawater and added using a sprayer to the surface of the section (resulting in 2 mg organo-clay complex /cm^2 mat surface). The sectioned pond was left for 3 h to achieve a complete settling of the organo-clay complex onto the mat surface at the bottom of the pond before sampling.

Two controls were used, the first was a section of the pond inoculated with mat material and medium without organo-clay complex and was used to monitor changes in the microbial community in the absence of the petroleum model compounds. The second control was a glass aquarium (59 x 26 x 30 cm, l/w/h) with medium, sterilized sea sand, but without mat material. It was used to check for photooxidation of the model compounds. The aquarium control was placed in an empty pond section, and both controls were under the same field conditions.

The experiment was performed for 100 days, and three homogeneous-looking sites on the mat surface were selected for sampling. Samples (3.3 cm^2 each) used for chemical analysis were taken from the three sites after 3 h and every 4 days during the first two weeks and every 10 days until the end of the experiment. For molecular microbiology analysis, 3 samples (4 cm^2 mat core each) were taken before the contamination and another 3 samples at the end of the experiment.

2.3.7 Recontamination experiment with intact mats in the sectioned pond

Winter experiment

A second experiment started on 30th October 2001 in order to investigate the effect of repeated exposure of the mat organisms to contaminants and the performance of the mats during winter time. It lasted until 8th February 2002. The same procedure as described above for the summer experiment was repeated in winter. The same amount of organo-clay complex was added to the same pond after the 100 days period of the summer experiment.

Following the appropriate procedure described above (summer experiment), the remaining empty sections were filled with sea water and inoculated with bacterial mats from the reference pond for adaptation for further contamination experiments. Water depth, temperature, salinity and pH values were also monitored three times per week.

2.4 Analytical procedure

2.4.1 Sample preparation

Drying and homogenation of sediment samples

Wet sediment samples weighing between 23 and 88 g were kept in separate glass beakers covered with aluminium foil punctured with small holes. The samples were kept in a deep freezer at -23°C over night. Then the samples were transferred to a freeze-dryer, and remained there for 4 days; the vacuum was 0.52 mbar and the temperature 4°C . The dried sediments were ground and homogenized for extraction. Figure 2.7 summarizes the analytical procedures by which the sediment samples were processed.

2.4.2 Ultrasonic extraction

The dried sediment samples weighed between 15.1 and 58.5 g. Each sample was transferred to a 100 ml beaker. A 30 ml mixture of dichloromethane and methanol (99:1, v/v) was added to each beaker and agitated ultrasonically for 15 min. The extractable organic matter was separated over a membrane filter. This procedure was repeated three times with 20 ml of the solvent mixture. The combined extracts were collected, and the solvent was reduced by rotary evaporation (40°C, 650-700 mbar) to a volume of about 1 ml. The extractable organic matter was then transferred to a small glass vial (ca. 2 ml volume), evaporated to dryness by a stream of dry nitrogen at ca. 30°C, weighed and then stored in a refrigerator at 4°C prior to further separation and derivatisation.

2.4.3 Precipitation of asphaltenes

The extractable organic matter (EOM) was transferred to a flask (ca. 100 ml volume). To dissolve the EOM and to precipitate the asphaltenes, 500 µl dichloromethane and 20 ml *n*-hexane were added. The *n*-hexane-soluble organic matter was filtered over anhydrous Na_2SO_4 (Fluka). The solvent was reduced by rotary evaporation (40°C, 300-400 mbar) to a volume of about 1 ml. The *n*-hexane-soluble fraction (bitumen) was then transferred to a small glass vial (ca. 2 ml volume) and evaporated to dryness by a stream of dry nitrogen at ca. 30°C.

2.4.4 Internal standards

Squalane, anthracene, mono-unsaturated C_{22} fatty acid and 5α-androstan-3β-ol were added to each bitumen sample and used as internal standards (ISTD) for quantification of the identifiable components.

2.4.5 Separation of the bitumen by column chromatography

The *n*-hexane-soluble fractions were separated into fractions of aliphatic hydrocarbons, aromatic hydrocarbons and polar heterocompounds (NSO fraction) by chromatographic separation using an MPLC system (Medium Pressure Liquid Chromatography; Radke et al., 1980).

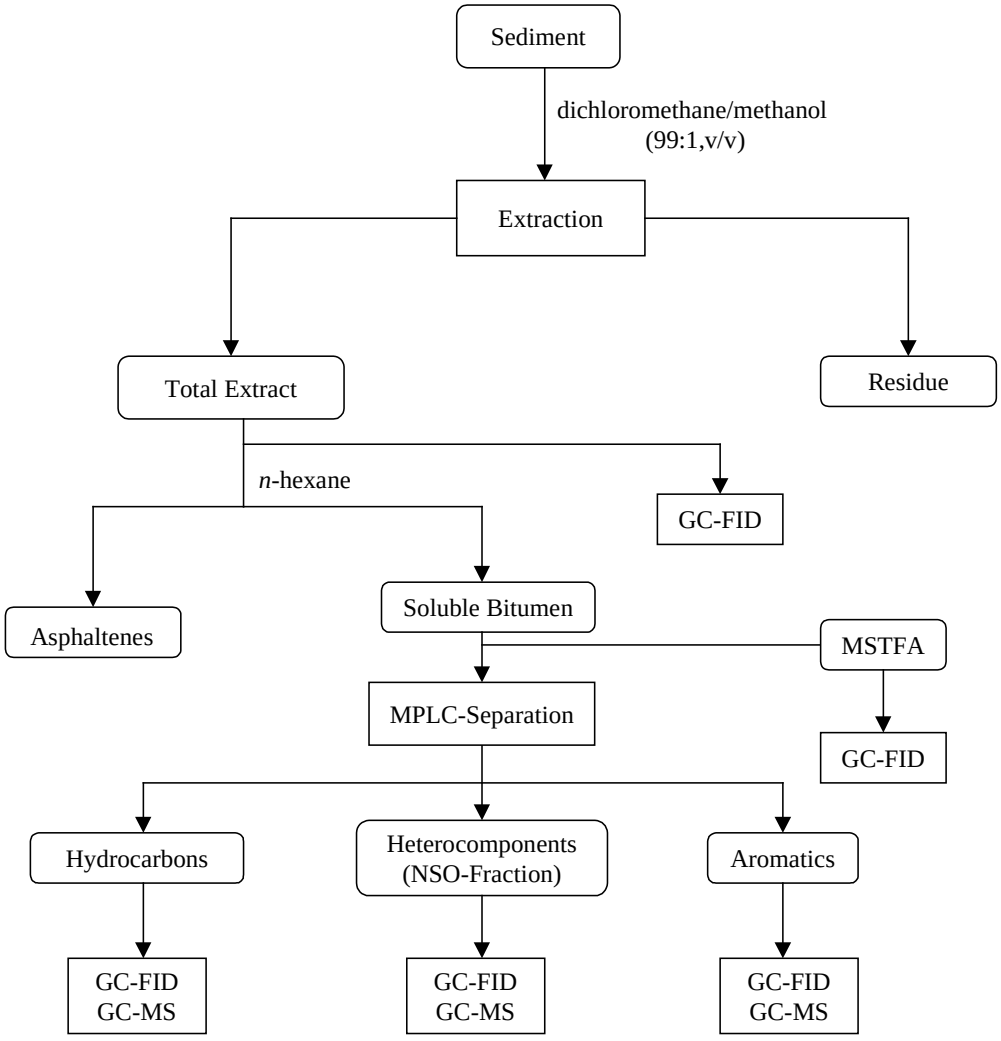


Figure 2.7. Analytical scheme for extraction, separation and analysis of lipids in the Wadi Gaza samples. MPLC = Medium pressure liquid chromatography; GC = Gas chromatography; MS = Mass spectrometry; FID = Flame ionization detection; MSTFA = N-Methyl-N-trimethylsilyl-trifluoroacetamide.

The MPLC system consists of an injection system with 16 sample tubes and 16 precolumns (100 mm x 10 mm ID; packed with Merck silica gel 100, deactivated at 600°C for 2 h) and a main column (250 mm x 10 mm ID; packed with Merck Lichoprep Si 60/40-63 µm). Separation was followed by a differential refractometer (RI detector) and a UV detector (absorption wave length = 259 nm). The RI detector records aromatic and nonaromatic hydrocarbons, and the UV detector specifically monitors the aromatic hydrocarbons**.** For all elutions super pure *n*-hexane was used. The aliphatic/alicyclic hydrocarbon fractions and the aromatic fractions were collected separately. The heterocompound fractions remained on the precolumn and were eluted with DCM/methanol (90:10%, v/v) after separation of the hydrocarbons. A 300 µl sample was injected into the sample loop, and the vial rinsed four times with 200 µl *n*-hexane and injected again. The nonaromatic and aromatic hydrocarbons passed the precolumn and reached the main column. The aliphatic/alicyclic hydrocarbons eluted at a rate of 8 ml/min and within 4 min and 15 s. The flow direction was reversed and the flow rate increased to 12 ml/min for 9 min and 15 s to elute the aromatic hydrocarbons. After separation, elemental sulfur was removed from the aliphatic hydrocarbon fractions by treatment with copper filings.

2.4.6 Derivatisation

For better gas chromatographic resolution, all polar fractions were trimethylsilylated prior to analysis by adding 100 µl MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) along with 100 µl dichloromethane to the fractions in the glass vials. The vials were allowed to stand for 90 min at 70°C.

2.4.7 Gas chromatographic analysis

The fractions were analyzed by gas chromatography on a Hewlett-Packard 5890 Series II instrument equipped with a temperature-programmed cold-injection system (Gerstel KAS 3), a flame ionization detector and a fused-silica capillary column (J&W DB-5, length = 30 m, inner diameter = 0.25 mm, film thickness = 0.25 µm). Helium was used as the carrier gas, and the temperature of the oven was programmed from 60°C (1 min isothermal) to 305°C (50 min isothermal) at a rate of 3°C/min. The injector temperature was programmed from 60°C (5 s hold time) to 300°C (60 s hold time) at 8°C/s.

2.4.8 Combined gas chromatographic/mass spectrometric analysis (GC/MS)

GC/MS studies were performed with the same type of gas chromatograph (helium as carrier gas) and a temperature program from 60°C (1 min isothermal) to 300°C (50 min isothermal) at a rate of 3°C/min. The gas chromatograph was coupled to a Finnigan SSQ 710B mass spectrometer operated at 70 eV. Structural assignments of individual compounds are based on comparison of relative gas chromatographic retention times and mass spectra with those reported in the literature.

Mass fragmentography was used extensively to characterize homologous and pseudohomologous series and to aid compound identification, particularly when coelution occurred. Quantification of individual components, when possible, was accomplished by measuring peak areas in gas chromatograms through automatic or, when necessary, manually modified automatic integration. Concentrations were calculated by comparison of the peak area of the natural compound with the area of the internal standard.

2.5 Chemical analysis of residual contaminants from degradation experiments

2.5.1 Slurry experiment with petroleum model compounds

Samples (2 ml) were taken from the Erlenmeyer flasks with disposable pipettes. The flasks were shaken vigorously to suspend the solid material in order to obtain homogeneous samples. The samples were extracted ultrasonically with a mixture of methanol, DCM and water (1:0.5:0.4, vol/vol/vol; modified after Bligh and Dyer, 1959). After centrifugation, the supernatant was collected in a separatory funnel. This procedure was repeated four times. DCM and water were added to the combined supernatant to give a methanol/DCM/H_2O ratio of 1:1:0.9 (vol/vol/vol), which resulted in phase separation. The DCM layer was collected, and the methanol-water phase was washed three times with DCM. The solvent of the combined DCM phase was removed with a rotary evaporator, and the extract was diluted to a concentration appropriate for gas chromatographic analysis.

The extracts were analyzed with a Hewlett-Packard 6890 gas chromatograph equipped with a Gerstel KAS3 temperature-programmable injector, a flame ionization detector, and a fused silica column (J&W DB-5HT; 30 m by 0.25 mm; film thickness 0.15 µm; carrier gas helium). After an isothermal phase at 60°C for 2 min, the oven was heated at a rate of 20°C/min to 150°C, then at a rate of 3°C/min to 310°C and then kept at 310°C for 15 min.

Model compounds were quantified by integration of the flame ionization detector signals and comparison with the signal of an internal standard (squalane) which was added immediately after extraction. The calculated initial amount of each model compound in a 2-ml sample was 66.67 µg. The abundance of the model compounds was determined relative to this value.

2.5.2 Slurry experiment with oil-clay complexes

At the time of sampling the flasks were shaken vigorously to suspend the solid material in order to obtain homogeneous samples. Samples (2 ml) were taken from the Erlenmeyer flasks with disposable pipettes. The samples were extracted and the extracts analyzed with the same methods as described for the slurry experiments with petroleum model compounds (section 2.5.1) but only with a different GC temperature program.

After an isothermal phase at 60°C for 2 min, the oven was heated at a rate of 10°C/min to 310°C and then at a rate of 20°C/min to 380°C. Crude oil components were quantified by integration of the FID signals and comparison with the signal of an internal standard (squalane) which was added immediately after extraction.

2.5.3 Slurry experiments with 2,4-D and terbutryn

Samples (2 ml and 5 ml) were taken from the Erlenmeyer flasks of the 2,4-D and terbutryn experiments, respectively, with disposable pipettes. The samples were stored at –23°C until analyses. The samples were transferred to a separatory funnel, and NaCl was added to quasi saturation. The 2,4-D samples were acidified with HCl (pH <2).

The 2,4-D and terbutryn samples were extracted in a separatory funnel four times with 2 ml and 3 ml DCM, respectively, by vigorous shaking. The solvent was removed from the combined extracts by a rotary evaporator, and the extract was diluted with DCM to a concentration suitable for gas chromatography. Squalane was added as an internal standard. The efficiency of the extraction procedure was tested with a 2,4-D standard mixture and yielded a recovery of more than 90% of the 2,4-D.

For GC analysis, 50 µl aliquots of the extract were transferred into a microvial and derivatized with MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide, 50 µl, 70°C for 90 min). The same GC as the one described above was used with a different temperature program. After an isothermal phase at 60°C for 2 min, the oven was heated at a rate of 10°C/min to 310°C (10 min isothermal).

The 2,4-D and terbutryn herbicides were quantified by integration of the FID signal and compared with that of the internal standard. Values were corrected for the different response factors of the 2,4-D and terbutryn derivatives compared to the internal standard.

2.5.4 Contamination and recontamination experiments with intact mats in the sectioned pond (petroleum model compounds)

Sampling of microbial mats

A 5 ml plastic syringe with 1.2 cm diameter was cut in order to have a big round opening. The syringe was gently inserted into the bottom of the pond to be filled with the top layer of the mat and sand and taken out. Only 1-2 ml (about 3.3 cm^2) of the material in the syringe which represents the top layer of the mat and its medium was collected in a 20 ml glass scintillation vial. This process was repeated three times. The same procedure was conducted for two other sites in the same pond. The vials were sealed and stored in a refrigerator at –40°C. The samples were taken at time zero and every 4 days during the first two weeks and after 10 days until the end of the experiments. Water depth, temperature, salinity and pH of this section during sampling was recorded.

The control section pond underwent the same steps except for the addition of organoclay with model compounds. All the samples were extracted and analyzed with the same method as described above for the slurry experiments with petroleum model compounds. As the only difference, the following temperature program was used: After an isothermal phase at 60°C for 2 min, the oven was heated at a rate of 20°C/min to 120°C, then at a rate of 4°C/min to 250°C and then at a rate of 20°C/min to 380°C (5 min isothermal).

Model compounds were quantified by integration of the FID signals and compared with the signal of an internal standard (squalane) which was added immediately after extraction. The calculated initial amount of each model compound in a 3.3 cm^2 sample was 330 µg. The abundance of the model compounds was determined relative to this value.

3. Results and Discussion

3.1 Organic matter content and bulk composition of Wadi Gaza sediments

The amount of organic matter in recent sediment may vary considerably (e.g. Tissot and Welte, 1984). Therefore, the determination of only the amount of organic matter is insufficient for the identification of oil pollution in soils or sediments. Hence, it is important to isolate the extractable organic matter, to analyze it in detail and to differentiate allochthonous from autochthonous contributions in order to determine petrogenic pollution.

Nevertheless, the mere amount of extractable organic matter can be indicative for oil pollution, as is the case with the six sediment samples investigated in this study. The amount of extractable organic matter (EOM, mg/g dry sediment), the sum of the liquid chromatography fractions (mg/g EOM) and the percentage of aliphatic, aromatic and NSO compounds relative to the sum of the liquid chromatographic fractions for these samples are presented in Table 3.1. EOM ranges from 0.93 to 7.1 mg/g dry sediment. The sum of liquid chromatography fractions varies between 0.53 and 0.96 mg/g EOM. The percentages of aliphatic, aromatic and NSO compounds range from 22.7 to 44.2%, 0.96 to 14.9% and 44.9 to 68.6%, respectively. EOM and the sum of the liquid chromatography fractions in the sediments from the western part of Wadi Gaza are higher than in the sample from the eastern part (Table 3.1).

Table 3.1. Composition of organic matter in sediments from Wadi Gaza.

Code	ICBM sample number	Extractable organic matter, EOM (mg/g)	SLCF, mg/g EOM	Aliphatics (%)	Aromatics (%)	NSO compounds (%)
a	5515	1.37	0.95	44.2	0.96	54.8
b	5525	0.93	0.53	37.5	6.30	56.2
c	6392	3.30	0.94	25.0	10.6	64.4
d	6393	2.77	0.89	22.7	8.6	68.6
e	6394	7.10	0.96	40.2	14.9	44.9
f	6395	2.12	0.71	36.2	9.2	54.6

SLCF = sum of the liquid chromatography fractions. Samples a and b were collected in January, 2000, from the western (a) and eastern (b) parts of the Wadi Gaza. Samples c-f were collected in August, 2001 from the western Wadi Gaza.

3.2 Aliphatic hydrocarbons

The composition of the aliphatic hydrocarbon fractions of samples a and b is presented by their gas chromatograms in Figure 3.1a. The parameters calculated on the basis of the distributions and the abundance of the *n*-alkanes as well as the isoprenoids pristane (Pr) and phytane (Ph) of all investigated samples are presented in Table 3.2. The abundance of the *n*-alkanes in samples a, b, c and e (Table 3.2) is positively correlated with the total aliphatic hydrocarbon contents in the extractable organic matter (Table 3.1).

Table 3.2. Aliphatic hydrocarbons characteristics in Wadi Gaza sediment samples.

Code	ICBM sample number	Pr/Ph	Pr/ n-C_{17}	Ph/ n-C_{18}	AHC (µg/g)	UCM (µg/g)	NA (µg/g)	UCM/ NA
a	5515	0.83	0.65	2.85	277	257	20.2	12.5
b	5525	0.84	0.63	0.75	184	159	25.6	6.21
c	6392	0.66	0.65	1.03	162	136	27.2	5.0
d	6393	0.55	0.82	2.80	90.5	84.9	5.6	15.1
e	6394	0.89	1.39	1.46	1395	1220	175	7.0
f	6395	0.51	1.37	3.03	119	112	6.8	16.6

Pr: pristane; Ph: phytane; AHC: total aliphatic hydrocarbons; UCM: unresolved complex mixture; NA: *n*-alkanes; hydrocarbon contents in µg hydrocarbon/g dry sediment.

n-Alkanes and an unresolved complex mixture (UCM) dominate the aliphatic hydrocarbon fractions. Concentrations of aliphatic hydrocarbons, here defined as the sum of the resolved peaks plus an estimate of the UCM, in Wadi Gaza sediment range from 90.5 to 1395 µg/g dry sediment (Table 3.2). The lowest concentrations were found in samples d and f (90.5 and 119 µg/g, respectively).

The aliphatic hydrocarbon concentrations observed in this study are higher than those reported in sediments collected from open marine areas worldwide, such as in the western North Atlantic (1.3-5.5 µg/g; Farrington and Tripp, 1977), in southern New England (mean value of 0.7 µg/g; Venkatesan et al., 1987), in sediments collected from coastal areas under urban or fluvial influence in the western Mediterranean Sea (Ebro delta: 0.67-32.5 µg/g; Grimalt and Albaiges, 1990), in sediments from the Cretan Sea coastal zone (0.5-5.7 µg/g; Gogou et al., 2000), from the El-Max Bay of the Egyptian Mediterranean coast (4.5-10.3 µg/g; Abd-Allah and El-Sebae, 1995), and the coastal area of the Black Sea (10-153 µg/g; Wakeham, 1996). Higher values were determined for the coastal area of Barcelona (mean value 495.7 µg/g; Tolosa et al., 1996) and the coastal area off the city of Alexandria in the southeastern Mediterranean Sea (20.3-1356.3 µg/g; Aboul-Kassim and Simoneit, 1995).

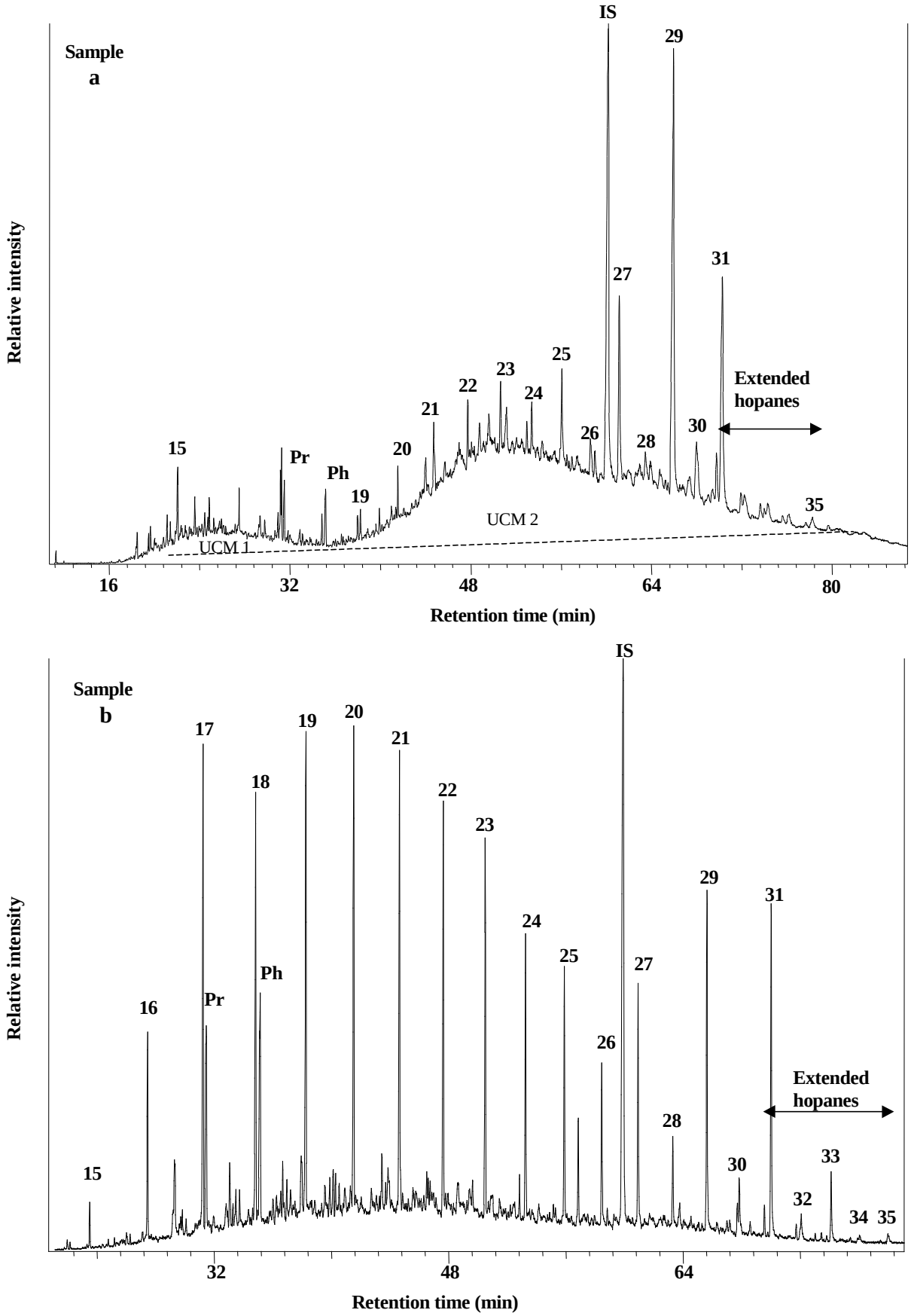


Figure 3.1. Representative gas chromatograms of two aliphatic hydrocarbon fractions extracted from Wadi Gaza sediments. Samples a and b were collected in January, 2000. Sample a (5515) is from the west and sample b (5525) from the east part of Wadi Gaza. Numbers indicate carbon numbers of *n*-alkanes. IS = internal standard, Pr = pristane, Ph = phytane, UCM = unresolved complex mixture.

The aliphatic hydrocarbon concentrations in the Wadi Gaza sediments are within the same range as those observed for the coastal area of the city of Alexandria.

3.2.1 n-Alkanes

Natural biological precursors of sedimentary organic matter generally contain a wide range of *n*-alkanes. These components particularly when of land plant origin, typically exhibit a strong predominance of odd carbon numbered homologues over those with even carbon number chain length (e.g. Meyers and Ishiwatari, 1993). Thermocatalytic (petroleum generation) processes which act on sedimentary organic matter after deep burial modify the *n*-alkane distribution so that no carbon number predominance remains (Hunt, 1979). This lack of an odd-over-even carbon number predominance can be used in environmental geochemistry to assist in identifying sediments contaminated with petroleum or its refinery products. Figure 3.2 shows histograms distribution patterns of the aliphatic hydrocarbon fractions extracted from all six Wadi Gaza sediment samples. The concentrations of *n*-alkanes vary between 5.6 and 175.4 µg/g dry sediment (Table 3.2), and the chain lengths range from 15 to 35 carbon atoms with distribution maxima occurring at *n*-eicosane (C_{20}) or *n*-nonacosane (C_{29}), but several of the distributions are bimodal and have a second, albeit smaller maximum. It is clearly evident that the *n*-alkane distributions represent a mixture of long-chain wax alkanes from higher land plants with a maximum at *n*-C_{29} or *n*-C_{31} and a strong odd-over-even carbon number predominance on the one hand and a petroleum-type component with a smooth distribution with respect to carbon number preference and maxima shifted to lower carbon numbers on the other hand. Whereas sample a is essentially dominated by plant wax alkanes, samples c and e show a strong dominance of petroleum *n*-alkanes. Samples b, d and f are of an intermediate type (Figure 3.2).

Table 3.3. *n*-Alkane characteristics in sediment samples from Wadi Gaza.

Code	ICBM sample number	CPI C_{27-33}	CPI C_{19-25}	CPI C_{17}	$\sum(C_{27}-C_{33})$ (µg/g)	Ter (µg/g)	Ter/NA (%)	Aqu (µg/g)	Aqu/NA (%)	*n*-C_{17} (µg/g)	*n*-C_{17}/NA (%)
a	5515	6.5	1.31	2.2	14.6	12.2	60	0.92	5	0.58	3
b	5525	3.1	1.01	1.3	7.92	5.3	21	4.11	16	1.91	7
c	6392	2.0	1.03	1.6	3.96	2.5	9	5.55	20	2.5	9
d	6393	2.5	1.00	1.8	2.45	1.5	27	0.52*	9*	0.29	5
e	6394	2.0	1.11	1.2	14.1	9.4	5	40.3	23	16.9	10
f	6395	2.2	1.05	1.7	1.72	1.1	16	0.86*	13*	0.33	5

CPI: carbon preference index; NA: total *n*-alkanes; $\sum(C_{27}-C_{33})$: sum of *n*-alkanes from heptacosane to tritriacontane**;** Ter: sum of terrestrial *n*-alkanes: heptacosane, nonacosane and hentriacontane; Aqu: sum of *n*-alkanes from aquatic organisms: pentadecane, heptadecane and nonadecane;
* heptadecane and nonadecane only (absence of pentadecane).

Carbon preference index (CPI) values (Bray and Evans, 1961) in the carbon number range of 19-25 are about 1.0 for all samples except for sample a which has a CPI value of 1.3 (Table 3.2), but -as said before- only a small petroleum component. All samples contain long-chain homologues (Σ *n*-C_{27}-C_{33} ranging from 1.7-14.6 µg/g dry sediment; Table 3.3 and Figure 3.2) with a long-chain odd-over-even carbon number preference reflected in high CPI values (CPI_{27-33} ranging from 2.0-6.5; Table 3.3). These long-chain homologues are known to be derived from higher plant waxes (Eglinton and Hamilton, 1967; Tulloch, 1976), and their occurrence reveals the importance of terrestrial material in the study area.

The sum of the concentrations of the three most abundant terrestrial *n*-alkanes (C_{27}, C_{29}, C_{31}) ranges from 1.1 to 12.2 µg/g dry sediment (Ter, Table 3.3), which accounts for 5-27% (except sample a, which has 60%) of the total *n*-alkane concentration. These are maximum number, because the contribution of petroleum alkanes to the homologues has not been deducted. The higher concentrations found in the sediments from the western part of the Wadi are due to the contribution of higher plants growing along the Wadi and the material transported into the Wadi by rain water run-off.

Higher and lower concentrations of terrestrial *n*-alkanes than those reported for Wadi Gaza sediments were found in coastal areas of the NW Mediterranean Sea receiving riverine discharges, e.g., up to 2.0 µg/g in the Ebro delta and up to 12.9 µg/g in the Rhône delta (Bouloubassi et al., 1997), whereas lower concentrations of terrestrial *n*-alkanes ranging from 0.028 to 0.42 µg/g in surface sediments of the Cretan Sea were reported by Gogou et al. (2000). The *n*-alkane homologues considered to be derived from aquatic organisms (defined

as the sum of the C_{15}, C_{17} and C_{19} *n*-alkanes) were smaller constituents in Wadi Gaza sediment, their summed concentrations range from 0.92 to 40.3 µg/g dry sediment, which accounts for 5-23% of the total *n*-alkanes (Table 3.3; again not corrected for petroleum contribution). Gogou et al. (2000) reported up to 7% short-chain *n*-alkanes in the Cretan Sea sediments. The high percentage found in the sediments from the western Wadi Gaza may partly be due to the massive growth of cyanobacterial mats and sea water intrusions in that area.

In cyanobacteria, *n*-heptadecane has been reported to be the most prominent *n*-alkane **(**Oró et al., 1967; Han et al., 1968a; Winters et al., 1969; Murata and Nishida, 1987). In Wadi Gaza sediments, the *n*-C_{17} alkane is a significant *n*-alkane homologue in nearly all aliphatic hydrocarbon fractions (Figure 3.2), and its abundance ranges from 0.3 to 17.0 µg/g dry sediment (Table 3.3). Köster et al. (1999) found ca. 2 µg/g dry weight of the C_{17} *n*-alkane in the cyanobacterium *Calothrix scopulorum*. The contribution by the cyanobacteria is reflected by high short-chain CPI values (CPI_{17}; Table 3.3), which range from 1.2 to 2.2.

3.2.2 Unresolved complex mixture

An unresolved complex mixture (UCM) was a major component of the aliphatic hydrocarbon fractions of the Wadi Gaza sediments, appearing in the gas chromatogram as a bimodal hump in the *n*-C_{15} to *n*-C_{35} range (UCM1 and UCM2; Figure 3.1a). It consists of a complex mixture of branched aliphatic hydrocarbons (Gough and Rowland, 1990) and has a well-known linkage to biodegraded petroleum residues (Brassell and Eglinton, 1980; Farrington and Quinn, 1973). Laureillard et al. (1997) and Venkatesan and Kaplan (1982) suggested that the UCM in the lower molecular weight range (UCM1; Figure 3.1a) can result from reworking of algal material.

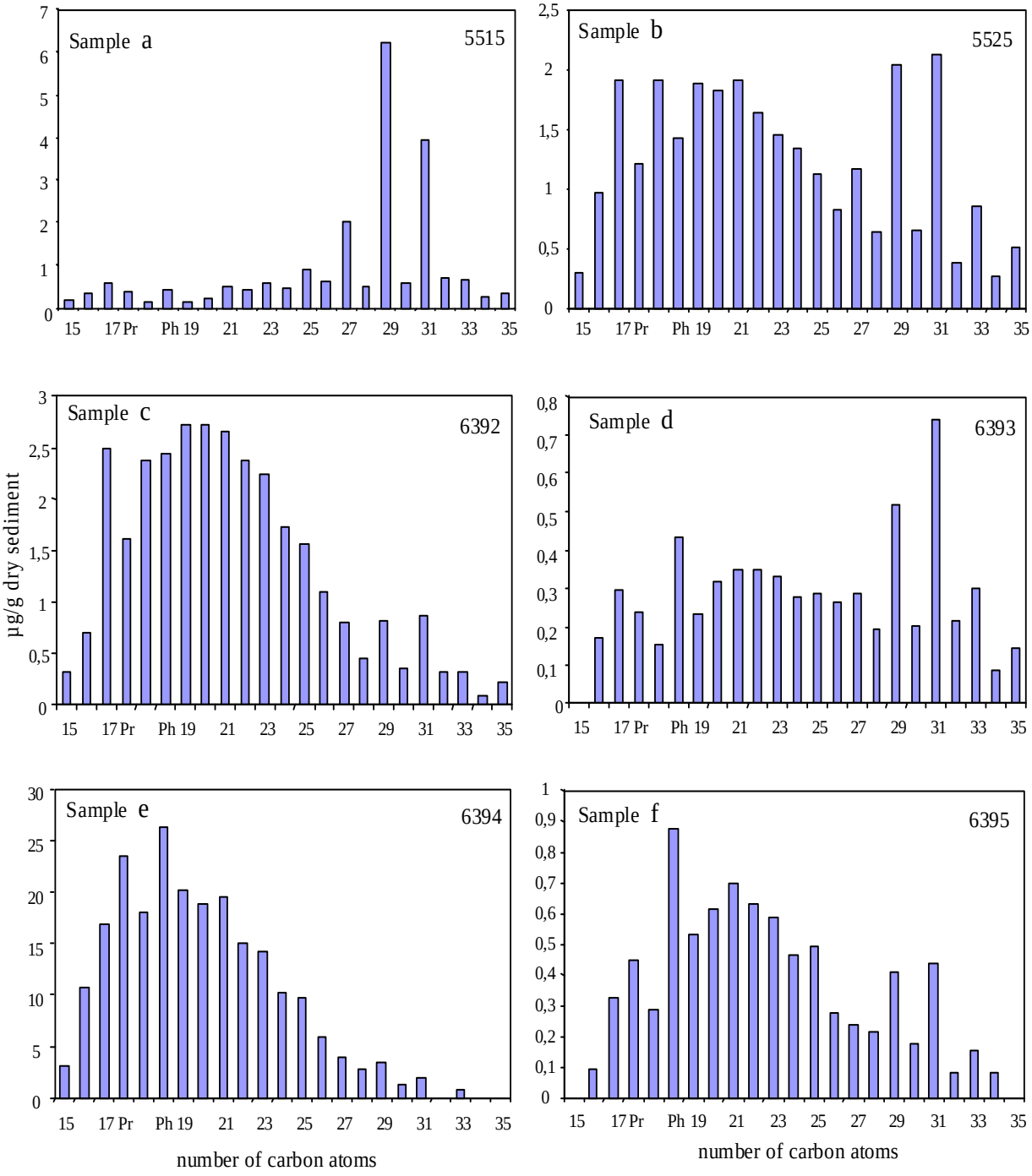


Figure 3.2. Distribution of *n*-alkanes in extracts from Wadi Gaza sediments samples. Samples a and b were collected in January, 2000 from the western and from the eastern Wadi Gaza, respectively. Samples c-f were collected in August, 2001 from the western part of Wadi Gaza. Pr: pristane; Ph: phytane. Samples b-f clearly indicate the presence of petroleum derivatives by a smooth envelope with no carbon number predominance. Samples a, b and d clearly indicate the presence of higher-plant material by the characteristic pattern of the long-chain homologues.

The high abundance of the UCM in all samples (85-1220 µg/g; Table 3.2) is a positive indication of chronic oil pollution. Additional evidence is provided by the ratio of the UCM to the *n*-alkanes (UCM/NA) which in most samples was between 5 and 16.6 (Table 3.2). This range characterizes hydrocarbon mixtures with significant contributions from degraded petroleum products (Simoneit, 1984; Simoneit and Mazurek, 1982). This is confirmed by the occurrence of specific biomarkers (e.g., hopanes; see Section 3.4 and Figure 3.5). The UCM concentrations in the Wadi Gaza sediments were much higher than those encountered in the open NW Mediterranean Sea (7-13 µg/g; Tolosa et al., 1996). But in coastal areas in the NW Mediterranean Sea near urban centers and major rivers, higher values (7-488 µg/g) were reported by Tolosa et al. (1996).

The spatial distribution of the UCM values indicates that more pronounced petroleum-related pollution occurred at the locations in the the western part of the Wadi where samples a and e were taken, although the *n*-alkanes in sample a do not confirm this, but they may already have been effectively biodegraded. The sample locations are situated close to urban and human activities. The values of the UCN/NA ratio are low in samples b and c, whereas higher values of this ratio were calculated for samples a, d and f, which are under the direct influence of anthropogenic activity of the local population.

The presence of mosquitoes in the Wadi Gaza area is one of several serious problems affecting the local population. The high level of petroleum-related pollution occurring in the eastern and western Wadi is due to the spraying of diesel oil and other refinery products to kill mosquito larvae in open sewage ponds and waters.

3.2.3 Pristane and phytane

The isoprenoid hydrocarbons pristane (Pr) and phytane (Ph) were present in all analyzed samples (Figure 3.2). The Pr/Ph ratios range from 0.51 to 0.89 (Table 3.2). The probable source of these two compounds is fossil fuel. The Pr/*n*-C_{17} and Ph/*n*-C_{18} ratios range from 0.63 to 1.39 and from 0.75 to 3.03 (Table 3.2), respectively.

Sample e (Figure 3.3) shows a smooth distribution of odd- and even-carbon-numbered *n*-alkane homologues (CPI = 1.1), an approximately equal abundance of phytane and pristane (Pr/Ph = 0.89), as well as a high abundance of pristane and phytane compared to the C_{17} and C_{18} *n*-alkanes (Pr/*n*-C_{17} = 1.39 and Ph/*n*-C_{18} = 1.46; Table 3.2). The gas chromatogram of the alkane fraction of sample e can be regarded as being typical for a crude oil with the microbial degradation process having already started (Volkman et al., 1983).

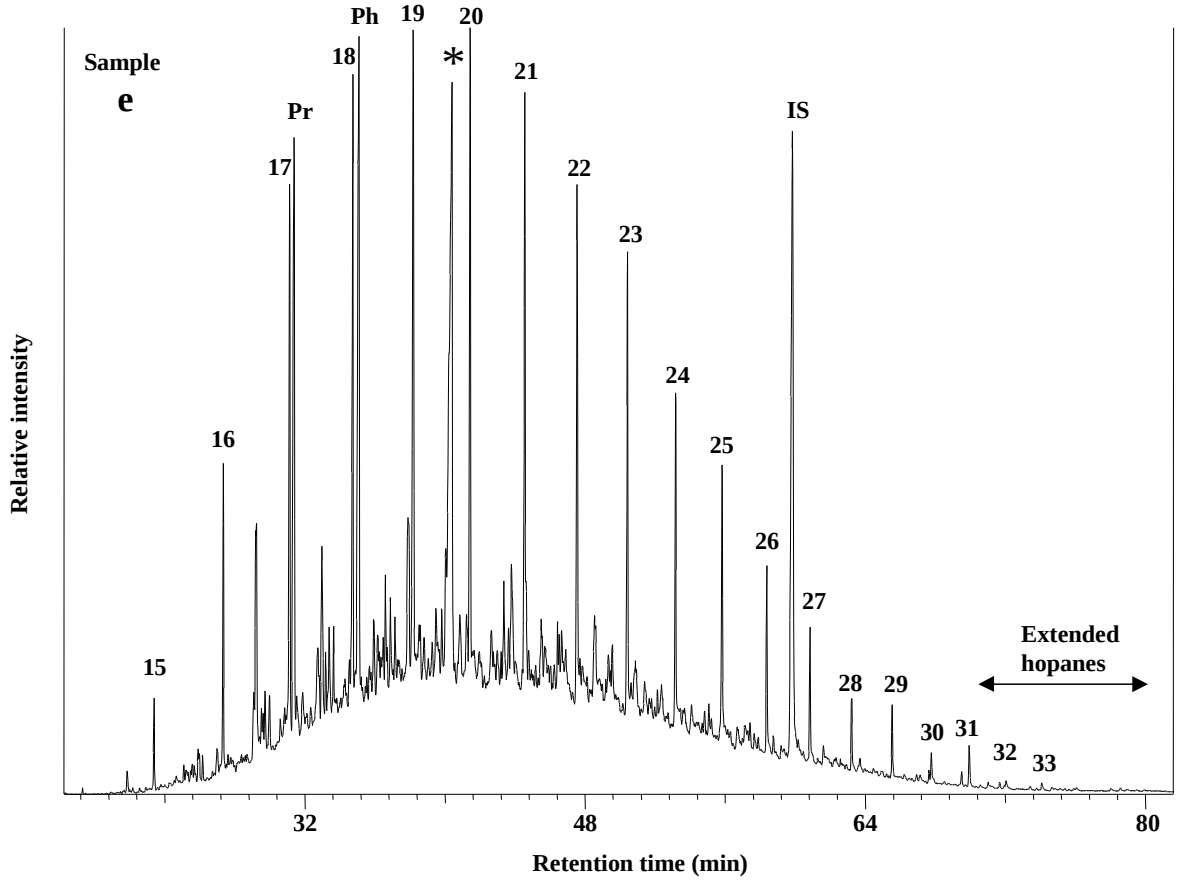


Figure 3.3. Gas chromatogram of the aliphatic hydrocarbon fraction extracted from sediment sample e from the west of Wadi Gaza. Numbers indicate carbon numbers of *n*-alkanes; *: Sulfur (S_8), incomplete desulfurization; IS: internal standard; Pr: pristane; Ph: phytane.

Samples c, d and f have lower Pr/Ph ratios than the aforementioned sample e (and also a and b) on the one hand, but they exhibit variably different Pr/*n*-C_{17} and Ph/*n*-C_{18} ratios on the other hand (Table 3.2).

Sample b (Table 3.2 and Figure 3.1) shows a uniform *n*-alkane distribution (CPI = 1.01), with phytane being more abundant than pristane (Pr/Ph = 0.84) and the C_{17} and C_{18} *n*-alkanes more abundant than pristane and phytane (Pr/*n*-C_{17} = 0.63 and Ph/*n*-C_{18} = 0.75).

The corresponding gas chromatogram also represents a typical saturated hydrocarbon fraction of a crude oil. In samples a, d and f, the higher abundance of phytane, compared to the C_{18} *n*- alkane (Ph/C_{18} = 2.85, 2.80 and 3.03, respectively) appears to be due to the degradation of the *n*-C_{18} alkane (and the other homologues) mainly by cyanobacterial mats. Safi et al. (2001) and Abed et al. (2002) reported the laboratory culture degradation of the C_{18} *n*-alkane by cyanobacterial mats which originated from the same sampling sites as the sediments. In samples e and f the higher abundance of pristane, compared to the C_{17} *n*-alkane (Table 3.2) is due to microbial degradation of the *n*-C_{17} alkane. The fact that the C_{17} and C_{18} *n*-alkanes are more abundant than pristane and phytane in sample b ($Pr/n\text{-}C_{17}$ = 0.63 and $Ph/n\text{-}C_{18}$ = 0.75), the only sample from the east of Wadi Gaza, is assumed to be due to an input of the saturated hydrocarbons from a different source.

Hence, besides the already mentioned parameters (organic matter content, bulk extract composition; Table 3.1), the saturated hydrocarbon distribution pattern is a reliable proof for the presence of oil-type pollutants in Wadi Gaza sediments.

3.2.4 Steranes and hopanes

Steranes and hopanes are polycyclic alkanes representing biological markers with highly characteristic distributions of structural and sterochemical isomers in oils and sediments with mature organic matter (Tissot and Welte, 1984; Waples 1985; Peters and Moldowan, 1993). Steranes are widely used to fingerprint petroleum (e.g. Seifert and Moldowan, 1986). The main steranes extend from C_{27} to C_{29} (Figure 3.4). The fossil fuel origin of these substances is evidenced by several features: In sedimentary rocks, sterane derivatives are formed by transformation of biological sterol precursors with biogenic sterical configuration sterols (14α,17α-20R) leading to the predominance of sterenes in shallow sediments, then steranes at intermediate depths. At higher thermal stress in deep formations and in petroleum, steranes with various geogenic sterical configurations are formed as well as diasteranes (Lichtfouse and Rullkötter, 1994; Lichtfouse et al. 1994; Mackenzie et al., 1980, 1982). Sterane isomerization at C-20 in the C_{29} 5α,14α,17α-steranes causes the 20S/(20S+20R) epimer ratio to rise from 0 to about 0.5 (or 0 to 50%) with increasing maturity (Seifert and Moldowan, 1986).

All the analyzed samples contained steranes and hopanes with distributions typical for oils (Figures 3.4 and 3.5). In Wadi Gaza sediments, the ratio of the 20S/(20S+20R) is in the a range from 32 to 44% (Table 3.4). Rullkötter et al. (1985) reported 20S/(20S+20R) ratios ranging from 34 to 37% in oils and 33 to 38% in heavy oils from different depths

derived from Senonian age carbonate source rock in the Dead Sea area. Sterane isomerization at the C-14 and C-17 positions in the 20S and 20R C_{29} 5α,14β,17β-steranes causes the ββ/(ββ+αα) ratio to rise from zero to values of about 0.8 with increasing maturity (Seifert and Moldowan, 1986). The C_{29} ββ/(ββ+αα) sterane isomerization ratio in the studied sediments ranges from 69% to 74% (Table 3.4). These values compare well with ββ/(ββ+αα) ratios ranging from 70 to 77% in heavy oils and 76% in oil from different depths in the Dead Sea area (Rullkötter et al., 1985).

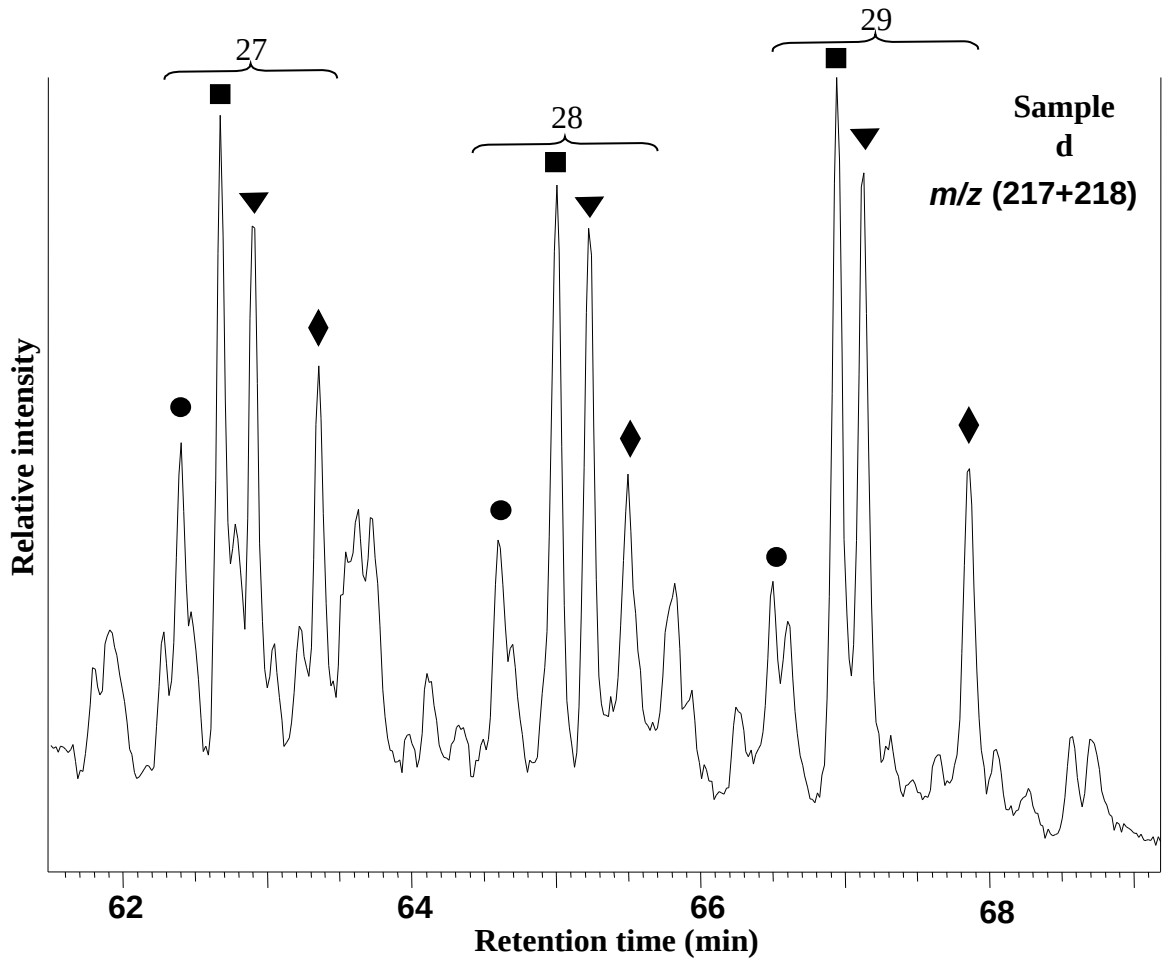


Figure 3.4. Sterane (*m/z* [217+218]) mass fragmentograms of the aliphatic hydrocarbon fraction of the extract from sediment sample d. ● = 20S-5α,14α,17α-steranes, ■ = 20R-5α,14β,17β-steranes, ▼ = 20S-5α,14β,17β-steranes, ♦ = 20R-5α,14α,17α-steranes.

Hopanes are found in almost all ancient sediments and crude oil (Seifert and Moldowan, 1986). A series of C_{27}-C_{35} pentacyclic triterpanes **(**17α,21β-hopanes) were identified in all sediment samples on the basis primarily of their mass spectra and GC retention times. Figure 3.5 shows the characteristic *m/z* 191 mass fragmentogram of sample c. Homologues with more than 29 carbon atoms exhibit the thermodynamically more stable

17α,21β configuration. The extended 17α-hopane series (C_{31}-C_{35}) occurred as 22S and 22R epimers (Figure 3.5). These patterns are characteristic of oil-derived hydrocarbons (e.g. Philp, 1985; Gogou et al., 2000; Jovančićević, 2003).

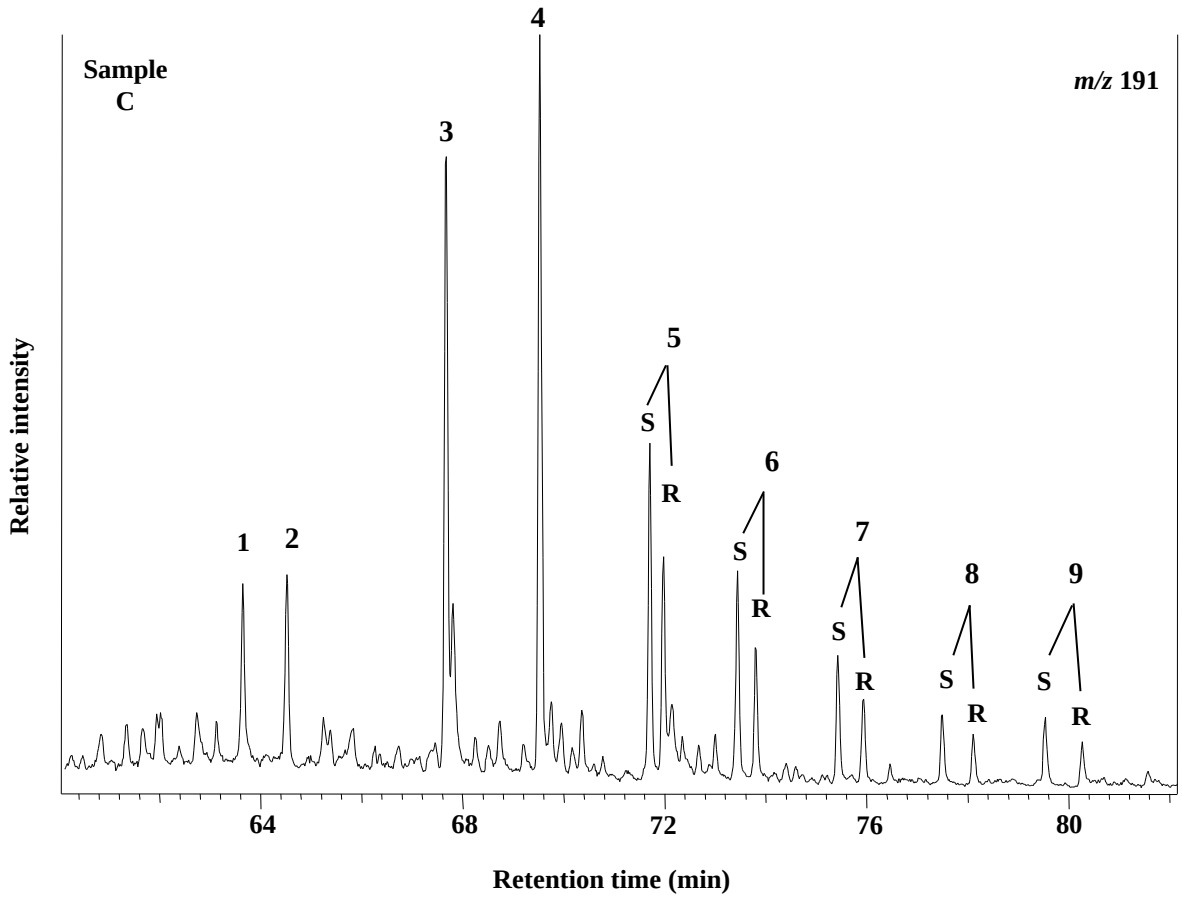


Figure 3.5. Hopane (*m/z* 191) mass fragmentogram of the aliphatic hydrocarbon fraction of the extract from sediment sample c. (1) 22,29,30-*trinorneo*-18α-hopane (Ts, $C_{27}H_{46}$); (2) 22,29,30-*trinor*-17α-hopane (Tm, $C_{27}H_{46}$); (3) 29-*nor*-17α,21β-hopane, ($C_{29}H_{50}$); (4) 17α,21β-hopane, ($C_{30}H_{52}$); (5) *homo*-17α,21β-hopane, ($C_{31}H_{54}$, 22S and 22R); (6) *dihomo*-17α,21β-hopane, ($C_{32}H_{56}$, 22S and 22R); (7) *trihomo*-17α,21β-hopane, ($C_{33}H_{58}$, 22S and 22R); (8) *tetrakishomo*-17α,21β-hopane, ($C_{34}H_{60}$, 22S and 22R); (9) *pentakishomo*-17α,21β-hopane, ($C_{35}H_{62}$, 22S and 22R).

17α-Hopanes found in sedimentary rocks are formed by the diagenesis of bacterial hopanepolyols (Ourisson and Albrecht, 1992). During sedimentary burial, the geochemically formed 17α,21β-22R-and S-hopane isomers gradually replaces the biogenic 17β,21β-22-R isomers (Lichtfouse and Rullkötter, 1994). Thus, the hopane fingerprint of Wadi Gaza sediments is typical of mature fossil fuels, as evidenced by the predominance of the geologically mature 17α,21β,22-S configuration over the corresponding 22-R configuration (equilibrium ratio 22S/(22S+22R) = 0.6). The ratio of the two C_{27} hopanes 18α-22,29,30-

trinorneo-hopane (Ts) and 17α-22,29,30-*trinor*-hopane (Tm) [Ts/(Ts + Tm)] is both maturity and source dependent. Moldowan et al. (1986) showed that the ratio can vary depending on organic facies. On the other hand, the ratio of Ts and Tm can be a sensitive indicator of thermal maturity when comparing oils or sediment samples from the same source (Ekweozor et al., 1979).

In the Wadi Gaza sediments, the ratio of Ts/(Ts + Tm) is in the range from 47 to 62% (Table 3.4). Rullkötter et al. (1985) reported Ts/(Ts + Tm) ratios ranging from 15 to 21% and 15 to 27% in oils and heavy oils from different depths, respectively. In Middle East oils the Ts/(Ts + Tm) ratio can vary from 0.28 in Kuwait crude oil to 0.33 in Arabian heavy crude oil and 0.6 in Arabian light crude oil (Volkman et al., 1997). The ratio observed in this study is within the range of those observed for Arabian light crude oil.

Table 3.4. Biological marker compound ratios for Wadi Gaza sediments

Code	ICBM Sample number	Steranes		Hopanes		
		20S/(20S+20R) [1] (%)	ββ/(ββ+αα) [2] (%)	Ts/(Ts+Tm) [3] (%)	22S/(22S+22R)[4] (%)	22S/(22S+22R)[5] (%)
a	5515	43	71	51	55	63
b	5525	44	74	53	61	61
c	6392	35	70	50	61	60
d	6393	39	69	62	61	60
e	6394	32	71	53	62	62
f	6395	39	72	47	62	61

[1] C_{29} 14α,17α-steranes (*m/z* 217)
[2] C_{29} 5α,14β,17β and 5α,14α,17α-steranes (*m/z* 217)
[3] C_{27} 18α(H)-*trinorneo*-hopane (Ts) and 17α-*trinor*-hopane (Tm) (*m/z* 191)
[4,5] C_{31} and C_{32} 17α,21β-hopanes (*m/z* 191)

The C-22 epimer ratios of C_{31} and C_{32} 17α,21β-*homo*-hopanes calculated for Wadi Gaza sediments range from 55 to 62% and 60 to 63% (Table 3.4). C_{31} epimer ratios of 56% were found in sewage sludge samples contaminated with fossil fuel from Nancy (Payet et al., 1999). Rullkötter et al. (1985) reported that C_{32}-22S/(22S + 22R) ratio ranged from 57 to 61% and from 60 to 63% in oil and heavy oil, respectively, from different depths in the Dead Sea area. The C_{32} epimer ratio observed in this study is within the same range as that observed for the heavy oil by Rullkötter et al. (1985).

Geological maturity parameters [Ts/(Ts + Tm) and C_{31} 22S/(22S + 22R)] of Wadi Gaza sediments (Table 3.4) yield average values typical of organic matter which has undergone a high thermal stress during geological times (Seifert and Moldowan, 1978). Therefore, the occurrence of mature hopanes in sediments clearly confirms the presence of a fossil fuel contamination as already suggested by the presence of the UCM as well as that of pristane and phytane.

3.3 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants throughout the environment (Suess, 1976; Blumer, 1976; Harvey, 1996). Combustion of organic material such as fossil fuels gives rise to PAHs by step-wise free radical mechanisms. Combustion-derived PAHs are dominated by the unsubstituted parent hydrocarbons, whereas PAHs in petroleum are dominated by the alkylated homologues (Laflamme and Hites, 1978). Once formed, PAHs are known to enter near-shore marine environments through the spillage of petroleum, industrial discharges, atmospheric fallout and urban run-off (Neff, 1979; Sharma et al., 1997; Gevao et al., 1998).

All Wadi Gaza sediments analyzed contained phenanthrene and its alkylated homologues (Figure 3.6). Four methylphenanthrene isomers were observed in all samples. Substituted phenanthrenes with methyl substituents in positions 1, 2, 3 or 9 have been reported to occur in sedimentary material including crude oil (Lekveishvili et al., 1980; Radke et al., 1993). Budzinski et al. (1995) reported that four methylphenanthrene isomers were determined in crude oil and rock extracts from different origins at various stages of thermal maturity. Radke et al. (1990) also found the four methylphenanthrene isomers in crude oils from the Handil field, Indonesia. Lichtfouse et al. (1997) found the four methylphenanthrene isomers in contaminated crop soil.

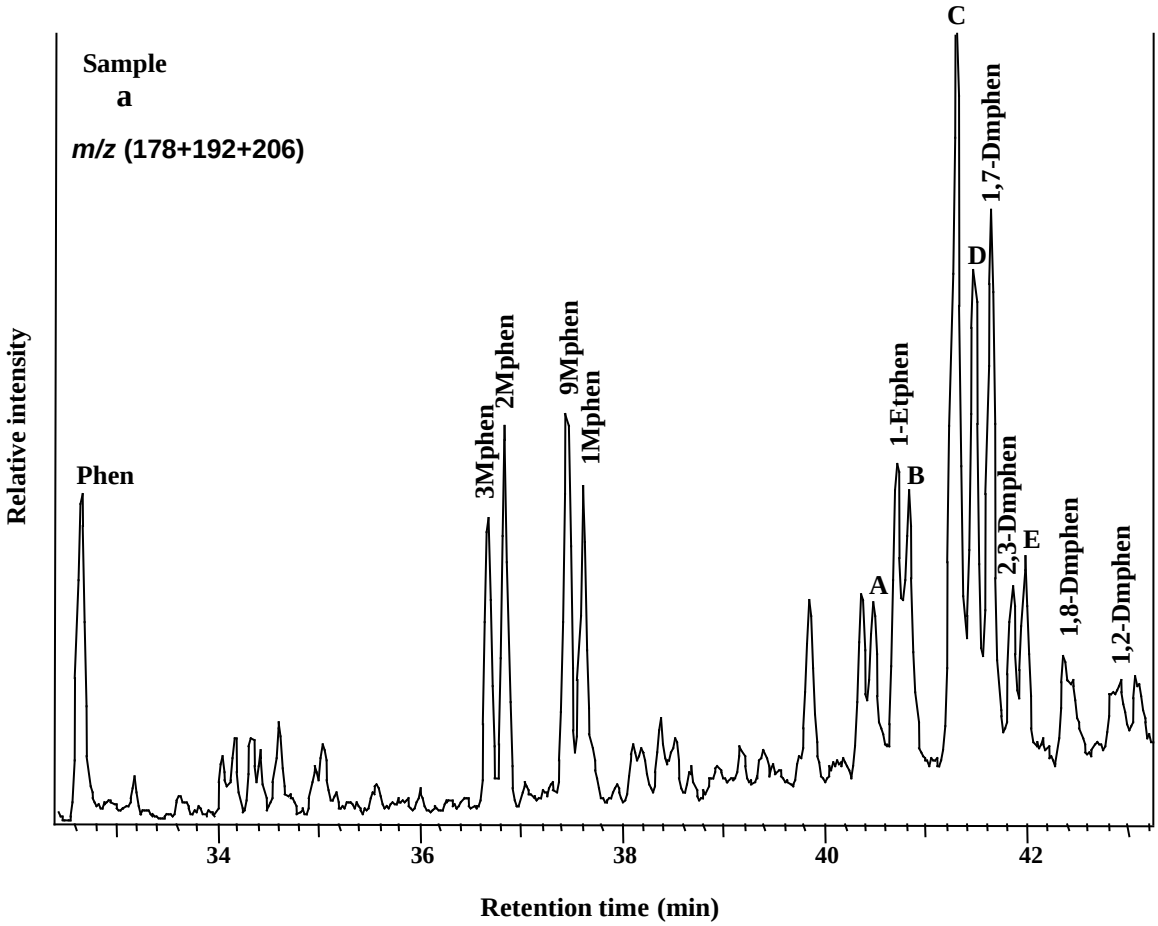


Figure 3.6. *M/z* (178+192+206) mass fragmentogram of the aromatic hydrocarbon fraction of the extract from sediment sample a showing alkylphenanthrene homologues and isomers. Phen = phenanthrene; Mphen = methylphenanthrene; Etphen = ethylphenanthrene; Dmphen = dimethylphenanthrene; A = 2-+9-Etphen+3,6-Dmphen; B = 2,6+2,7+3,5-Dmphen; C = 1,3+2,10+3,9+3,10-Dmphen; D = 1,6+2,5+2,9-Dmphen; E =1,9+4,9+4,10-Dmphen.

During the last 20 years, numerous parameters based on relative abundances of biomarker isomers of organic matter in sediments and petroleums were developed (Peters and Moldowan, 1993). The ratios of methylphenanthrenes to phenanthrene (Mphen/Phen) were examined as an indication of anthropogenic influence such as combustion of organic materials. These ratios are reported to be in the range of 0.5-1 in sediments dominated by phenanthrenes arising from combustion processes and from 2-6 in sediments dominated by fossil fuel phenanthrenes (Prahl and Carpenter, 1983). The ratios of Mphen/Phen was reported to be 4.0 for used crankcase oil (Pruel and Quinn, 1988), close to 1.0 for street and urban dust samples (Takada et al., 1990, 1991) and about 0.5 for atmospheric fallout (Takada et al., 1991). Notar et al. (2001) found Mphen/Phen ratios >2 in sediments from the Gulf of Trieste, Northern Adriatic Sea. The Mphen/Phen ratios are about 0.5-0.7 in sediments from San Francisco Bay, California, indicating that the dominant recent sources of PAHs are from combustion processes (Pereira et al., 1999). In the Wadi Gaza sediments the ratios are in the

range of 2.23-4.57 with the exception of sample f which has a ratio of 6.81 (Table 3.5). The Mphen/Phen ratios for all Wadi Gaza sediment is higher than two, which indicates fossil fuel sources.

Dimethylphenanthrenes occur in crude oils, oil shales, coals and in the bitumens and kerogens that are the sources of liquid petroleum (Yawanarajah and Kruge, 1994; Budzinski et al., 1995; Kruge, 2000). In all investigated sediments from the Wadi Gaza dimethylphenanthrenes were present (e.g. Figure 3.6). Dimethylphenanthrenes were also found in surface sediments from the Cretan Sea contaminated with petroleum products (Gogue et al., 2000).

Table 3.5. Concentrations of sterathiols and Mphen/Phen and 4-MDBT/1-MDBT ratios determined for Wadi Gaza sediments.

Code	ICBM sample number	Sterathiols µg/g dry sediment			∑Mphen/Phen ratio	4-MDBT/1-MDBT ratio
		C_{27}	C_{28}	C_{29}		
a	5515	3.6	0.4	0.7	2.23	2.2
b	5525	3.4	0.3	0.8	4.08	3.6
c	6392	18.4	1.2	1.6	3.22	2.2
d	6393	10.5	1.0	2.0	3.34	2.2
e	6394	9.1	0.01	2.9	4.57	3.4
f	6395	15	1.2	1.8	6.81	2.1

∑Mphen/Phen: sum of methylphenanthrenes/phenanthrene; 4MDBT/1 MDBT: 4-methyldibenzothiophene/1- methyldibenzothiophene ratio.

3.4 Aromatic sulfur compounds

Organic sulfur compounds such as benzothiophene, dibenzothiophene and their alkylated homologues are common constituents of sulfur-rich oils. Dibenzothiophene and its derivatives have been widely used as model organic sulfur compounds in petroleum studies (Kilbane and Bielaga, 1990). Alkyl dibenzothiophenes have been shown to be quite persistent in petroleum-contaminated environments (Hostettler and Kvenvolden, 1994; Wang et al., 1994). In all investigated Wadi Gaza sediments dibenzothiophene, methyl and ethyldibenzothiophene were found (Figure 3.7). Contaminated sediments from the Gulf of Lions (Mediterranean Sea, France) were found to contain the four methyldibenzothiophenes isomers (Domine et al., 1994).

Jiang et al. (1994) found 4-methyldibenzothiophene in marine sediments. 4-Methyldibenzothiophene (4-M-DBT) is thermodynamically more stable than 1-

methyldibenzothiophene (1-M-DBT). With increasing maturity, one observes a decrease in the amount of the less stable α-substituted 1-M-DBT isomer compared with the amount of the more stable β-substituted 4-M-DBT isomer (Radke et al., 1986, 1988). Because of this a methyldibenzothiophene index was defined as 4-M-DBT/1-M-DBT (Radke et al., 1986; Budzinski et al., 1993) and is known to increase with increasing maturity of the oil.

The methyldibenzothiophene index (MDI) of Wadi Gaza sediments ranges from 2.1 to 3.6 (Table 3.5). MDI values in the range of 2.0 to 5.0 were reported for five crude oils from different sources by Andersson and Sielex (1996). Chakhmakhchev et al. (1997) found a 4/1-MDBT ratio of 2.5 and attributed that to mature oil whereas a ratio of 15.4 indicated overmature oil. The MDI values of Wadi Gaza sediments are similar to the MDI values of the crude oils mentioned above.

The occurrence of methyldibenzothiophenes in the Wadi Gaza sediments clearly confirms their contamination with crude oil or petroleum derivatives.

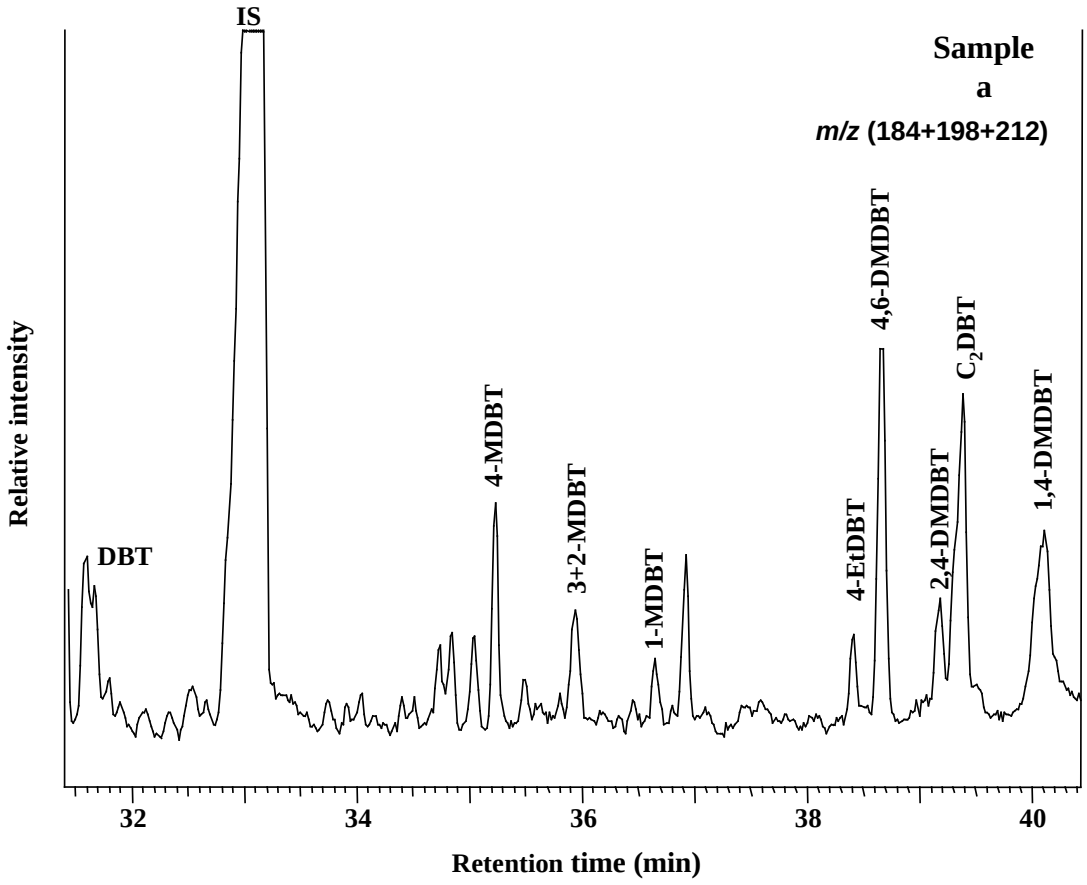


Figure 3.7. *M/z* (184+198+212) mass fragmentogram of the aromatic hydrocarbon fraction of the extract from sediment sample a showing alkyldibenzothiophene isomers. IS = internal standard; DBT = dibenzothiophene; MDBT = methyldibenzothiophene; EtDBT = ethyldibenzothiophene; DMDBT = dimethyldibenzothiophene.

Monoaromatic C_{27}, C_{28} and C_{29} and triaromatic $C_{21,}$ $C_{26,}$ C_{27} and C_{28} steroid hydrocarbons were present in the sediments. These compounds are biomarker components of fossil fuels. Besides their occurrence in crude oil and sediments with mature organic matter (e.g. Peters and Moldowan, 1993), triaromatic steroid hydrocarbons have been found as indicators for contamination in estuarine sediments (Killops and Howell, 1988) and in natural bitumen and pyrolysates of humic coals (Lu and Kaplan, 1992). The dominant source of the aromatic steroid hydrocarbons in the surface sediments of Wadi Gaza is probably from fossil fuels and petroleum derivatives containing these biomarkers.

3.5 Sterenes and sterathiols

Sterenes are among the numerous products formed during diagenetic and catagenetic transformation of biogenic sterols (Mackenzie et al., 1982). All aromatic hydrocarbon fractions of the Wadi Gaza sediment extracts contain C_{27}, C_{28} and C_{29} Δ^2-sterenes and minor relative concentration Δ^3-sterenes. The sterenes are dominated by the C_{27} homologues (Figure 3.8). In addition, the aromatic hydrocarbon fractions of the sediment extracts contain a series of late-eluting sulfur-bearing compounds (Figure 3.8). These were tentatively identified as 5α-stera-3β-thiols with C_{27}, C_{28} and C_{29} carbon skeletons on the basis of their mass spectra and gas chromatographic retention times in comparison with published information (Schaeffer et al. 1995; Adam et al. 1991; Summons and Capon, 1988). The sterathiols are dominated by the C_{27} homologue. The C_{27} sterol homologues are present in human and animals feces in higher concentrations than the C_{29} homologues (Leeming et al., 1997).

The prominence of C_{27} sterathiols in Wadi Gaza sediment is due to the presence of C_{27} sterols in the sediments which originate from untreated waste water discharge and release of animal feces into the study area. The occurrence of sterathiols has been described only for macromolecular organic matter (Schaeffer et al., 1995; Adam et al., 1991) and peats and rootlet-containing clay sediments in the coastal area of Northern Germany (Dellwig et al., 1998). The estimated concentrations of C_{27}, C_{28} and C_{29} sterathiols (Table 3.5) vary between 3.4 and 15, 0.01 and 1.2, and 0.7 and 2.9 µg/g dry sediment, respectively. The C_{29} sterathiols were estimated to occur in concentrations between 0.1 and 15 µg/g TOC in Holocene peat layers in the coastal area of the Northwest Germany (Scholz-Böttcher et al., 1999). The presence or absence of sterathiols is related to the occurrence of free reduced sulfur species which may react with sterenes in sediment to form 5α,3β-thiols.

Wadi Gaza sediments offer sufficient organic matter as a carbon source and hence ideal conditions for sulfate-reducing bacteria provided that sulfate is also available. A potential sulfate source in Wadi Gaza is sea water and possibly also untreated wastewater discharge. This implies that the sediments, which normally are under the influence of sulfate-poor freshwater, must occasionally have become brackish. Hence, the presence of sterathiols in the sediment can be taken as an indicator for marine incursions.

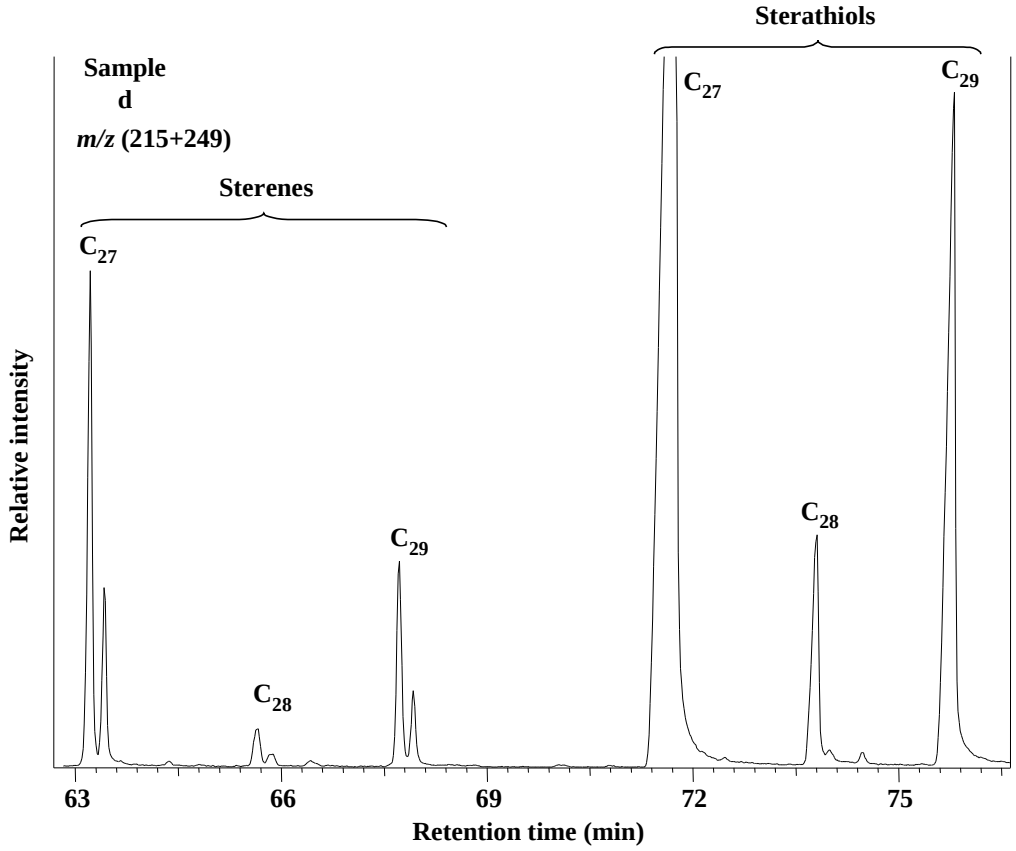


Figure 3.8. Sterene (*m/z* 215) and sterathiol (*m/z* 249) mass fragmentograms of the aromatic hydrocarbon fraction of the extract from sample d showing the distribution of sterenes and sterathiols.

3.6 n-Fatty acids

All *n*-hexane-soluble fractions of the six sediment extracts contain *n*-fatty acids with chain lengths of 14 to 22 and some >22 carbon atoms (e.g. Figure 3.9). As expected for a biosynthetic origin, the distributions are dominated by homologues with even carbon numbers. The most abundant fatty acid is palmitic acid ($C_{16:0}$). Unsaturated fatty acids have 16 or 18 carbon atoms with one of the monounsaturated *n*-$C_{18:1}$ isomers having the highest relative abundance in some samples. In addition, trace amounts of odd-carbon-numbered iso and anteiso $C_{15:0}$ acids were found.

The long-chain fatty acids ($\geq C_{22}$) reflect a contribution from higher terrestrial plants (Eglinton and Hamilton, 1967; Eglinton et al., 1973; Matsuda and Koyama, 1977) whereas the saturated and unsaturated fatty acids in the lower carbon number range are presumably derived mainly from algae (Cranwell, 1974; Sargent and Whittle, 1981; Shameel, 1990) with the exception of palmitic (*n*-$C_{16:0}$) and stearic acid (*n*-$C_{18:0}$) which are common both in microorganisms and in higher plant material. The detected iso and anteiso fatty acids are probably related to bacterial sources.

3.7 n-Alcohols

n-Alcohols in sediments can have different origins. All investigated samples contain *n*-alcohols with chain lengths ranging from C_{16} to C_{32}. An example of a gas chromatogram of an NSO fraction of the sediment extract from Wadi Gaza is shown in Figure 3.9.

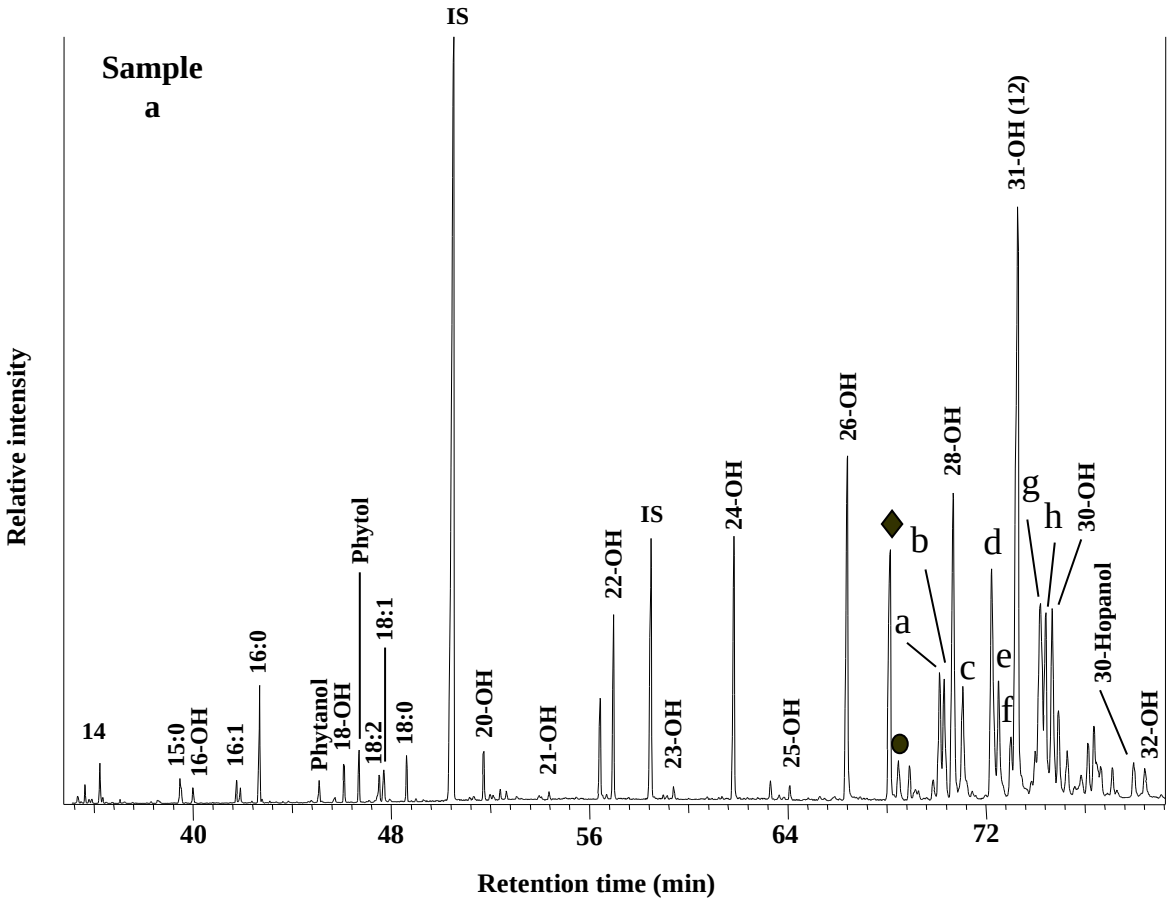


Figure 3.9. Representative gas chromatogram of an NSO fraction of the six sediment extracts (sample a). Numbers denote carbon numbers of *n*-fatty acids. Numbers-OH represent carbon numbers of *n*-alcohols. IS = internal standard. a = cholest-5-en-3β-ol, b = 5α-cholestan-3β-ol, c = 24-methyl-5α-cholesta-5,22-dien-3β-ol, d = 24-methylcholest-5-en-3β-ol + 24-ethyl-5β-cholestan-3α-ol, e = 24-methyl-5α-cholestan-3β-ol + 24-ethyl-5β-cholestan-3β-ol, f = 24-ethyl-cholesta-5,22-dien-3β-ol, g = 24-ethyl-5α-cholest-5-en-3β-ol, h = 24-ethyl-5α-cholestan-3β-ol, ♦ = coprostanol: 5β-cholestan-3β-ol, ● = epicoprostanol: 5β-cholestan-3α-ol.

The distributions are dominated by homologues with even carbon numbers. Distribution maxima occur at *n*-hexacosan-1-ol (C_{26}). A distribution pattern with a predominance of tetracosan-1-ol (C_{24}), hexacosan-1-ol (C_{26}) and octacosan-1-ol (C_{28}) is characteristic for land plant material origin (Harwood and Russell, 1984; Cranwell, 1988). The mid-chain funtionalized hentriacontan-12-ol (C_{31}) is most abundant *n*-alcohol in some samples.

3.8 Steroid alcohols

In Figure 3.9, the gas chromatogram of the NSO fraction also shows the elution range of steroidal alcohols. Compound identification is based on relative retention times and comparison with mass spectra from the literature (Budzikiewicz, 1972; Brassell, 1980; McEvoy, 1983). The sterols are dominated by 24-ethylcholest-5-en-3β–ol [g] followed by its saturated analogue, 24-ethyl-5α-cholestan-3β–ol [h], and 24-methylcholest-5-en-3β–ol [d]. All three sterols and the next most abundant sterol, 24-ethylcholesta-5,22-dien-3β–ol [f], are mainly derived from higher terrestrial plants (Huang and Meinschein, 1976, 1979; Marsh et al., 1990). The C_{28} sterol 24-methylcholesta-5,22-dien-3β–ol [c] is biosynthesized by higher land plants as well as planktonic organisms (Patterson, 1970; Idler and Wiseman, 1971; Marsh et al., 1990), whereas cholesterol [a] and its saturated analogue, 5α-cholestan-3β-ol [b], are ubiquitous in sediments and thus are not source indicative (Mackenzie et al., 1982).

3.9 Specific markers

The major source of phytol is chlorophyll a and already released from it by hydrolysis in the water column (Baker and Louda, 1983). Due to the highly reactive allylic hydrogen atoms, phytol is rapidly degraded by oxidation (Rontani et al., 1990). Phytol was found in all investigated samples (e.g. Figure 3.9).

A very specific organic tracer is coprostanol (5β-cholestan-3β-ol), which is produced in the digestive tract of humans by the anaerobic microbial hydrogenation of the double bond in cholesterol (cholest-5-en-3β-ol). Coprostanol is an excellent marker for sewage pollution. It has been proposed as a measure of fecal pollution by a large number of researchers (e.g. Walker et al., 1982; Sherwin et al., 1993; Bethell et al., 1994; Leeming et al., 1997; Réveillé et al., 2003). It has a very long persistence time (Bethell et al., 1994; Bull et al.,1998).

All analyzed sediments from the eastern and western parts of Wadi Gaza were found to contain fecal sterols (Figure 3.9 and Table 3.6). Leeming et al. (1997) found that the major

sterols in human feces are coprostanol, cholesterol and 24-ethylcholesterol. C_{27} sterols were present in human feces in higher concentrations than C_{29} sterols, whereas the opposite was observed for sheep (herbivores).

When fecal matter is discharged or washed into aquatic systems, the sterol fingerprint of the source animal is diluted and mixed with the sterol profile of autochthonous algae, detritus and other material. Despite this, even low concentrations of fecal sterols can indicate whether fecal pollution from humans or herbivores is present. Experiments on sewage effluents indicate that there is no significant difference in the degradation rate of C_{27} and C_{29} sterols or stanols over a period of 2-3 weeks (Leeming, unpublished work cited in Leeming et al., 1997), suggesting that the sterol profile of fecal pollution would remain intact in the environment.

Coprostanol was present in significantly higher concentrations of up to 20 µg/g in the Wadi Gaza sediment samples collected in August 2001 relative to those found in sediments collected in January 2000 (<3 µg/g; Table 3.6). Based on the coprostanol concentration data, the highest levels of sewage-derived organic matter were detected in sediments from the western part of Wadi Gaza. Coprostanol concentrations ranging from 0.1 to 1.25 µg/g in sediments from the Southeast Atlantic Ocean and the Gulf Coasts were reported by the Benthic Surveillance Project of the NOAA Status and Trends Program (NOAA, 1984). Coprostanol concentrations in sediments have been reported to range from <0.01 to 4.8 µg/g in Biscayne Bay (Pierce and Brown, 1987) and up to 3.7 µg/g in the St. Johns River Basin (Pierce et al., 1988). Coprostanol contents of the Sydney inner-shelf sediments ranging from 0.004 to 1.91 µg/g were reported by Leeming et al. (1997). In Key West sediments coprostanol is present at a level of 0.03 to 0.34 µg/g dry and up to 4.85 µg/g in the Weeki Wachee River (Sherblom et al., 1997). Wadi Gaza sediments had variable contents (1.62 to 20.09 µg/g), whereas in Kaohsiung Harbour, Taiwan, sewage-impacted values of 0.58 to 128 µg/g were reported by Jeng and Han (1994), and in the canals and lagoons of Venice, Italy, which contain untreated sewage, the values range from (1 to 41 µg/g).

3.9.1 Human and herbivore contributions

Leeming et al. (1997) suggested that it is possible to estimate the relative contributions of fecal pollution by herbivorous animals (e.g. cows and sheep) and humans by comparing the content of coprostanol and 24-ethylcoprostanol by using the following mathematical term:

$$\left(\frac{5\beta\text{-}C_{27}\Delta^0}{5\beta\text{-}C_{27}\Delta^0 + 5\beta\text{-}C_{29}\Delta^0} \right) \times 100$$

where 5β-$C_{27}\Delta^0$ is coprostanol and 5β-$C_{29}\Delta^0$ is 24-ethylcoprostanol. If the term is >73 then contamination probably originates solely from humans. If the value is <38 then the fecal contamination probably originates solely from herbivores.

In the case of a sample containing 45% coprostanol (relative to the sum of coprostanol and 24-ethylcoprostanol), a factor of 2.86 (derived from the difference between the mean maximum percentages measured of the two sources divided by 100 [(i.e. 73-38)/100] can be added for every percent below 73% to estimate the proportion of human fecal contamination relative to herbivores. In that instance, (73-45) x 2.86 equals 80% herbivore contribution and, therefore, 20% human contribution.

Table 3.6. Coprostanol concentrations (µg/g dry sediment) in sediments from Wadi Gaza.

Code	ICBM Sample number	Coprostanol (c)			$C_{27}/C_{27}+C_{29}$ (%)	Human (%)	Herbivores (%)	Epicoprostanol (e)	c/e ratio
		C_{27}	C_{28}	C_{29}					
a	5515	2.58	0.32	2.21	54	46	54	0.22	11.52
b	5525	1.62	0.17	1.31	55	49	51	0.14	11.41
c	6392	10.58	1.09	8.14	57	54	46	1.22	8.65
d	6393	13.10	1.39	9.35	58	57	43	1.02	12.86
e	6394	20.09	2.03	12.46	62	69	31	2.57	7.82
f	6395	3.39	0.44	2.09	62	69	31	0.36	9.44

C_{27}, coprostanol; C_{28}, 24-methylcoprostanol; $C_{29,}$ 24-ethylcoprostanol.
Samples a and b were collected in January, 2000 from the western and from the eastern part of Wadi Gaza, respectively. Samples c-f were collected in August, 2001, from the western Wadi Gaza.

Based on the coprostanol and 24-ethylcoprostanol contents shown in Table 3.6, application of the equations above provides the information that the herbivore contributions to the sediments in the western part of Wadi range from 31 to 54%, whereas 46-69% are from human contributions. In the eastern part of the Wadi 51% is contribution from herbivores (Table 3.6 and Figure 3.10). The contributions of the herbivores and humans at both sites are due to the agricultural grazing and the intense untreated wastewater discharge, respectively.

Venkatesan et al. (1986) and Venkatesan and Kaplan (1990) suggested that the ratio of coprostanol to epicoprostanol (c/e ratio) can be used to distinguish inputs of domestic waste from material of non-human origin. They suggested that humans-related waste will result in a ratio which is close to or larger than one (greater proportion of coprostanol). Lower values for the ratio would indicate that the dominant source are marine mammals. Human fecal matter contains only trace amounts of epicoprostanol. In Wadi Gaza sediments epicoprostanol was only a minor component, three samples contained less than 10% and the other three samples <13% relative to coprostanol (Table 3.6). Leeming et al. (1997) found that in the Sydney inner-shelf sediments epicoprostanol was <10% of coprostanol. These data indicate that marine mammals do not contribute significantly to this environment.

The c/e ratio can vary between samples from wastewater treatment facilities. Eganhouse et al. (1988) reported coprostanol and epicoprostanol concentrations in sludges from six treatment plants using different treatment methods. The c/e ratios for these six sludges ranged from 0.9 to 9.0 with the anaerobically digested sludge having a c/e ratio of 0.9 (Eganhouse et al., 1988). The c/e ratio of Key West sediments range from 0.2 to 9.0 and those of Sarasota Bay sediments from 0.2 to 1.5 (Sherblom et al., 1997). The c/e ratios of Wadi Gaza sediments range from 7.82 to 12.86 with most values exceeding the value of 8.

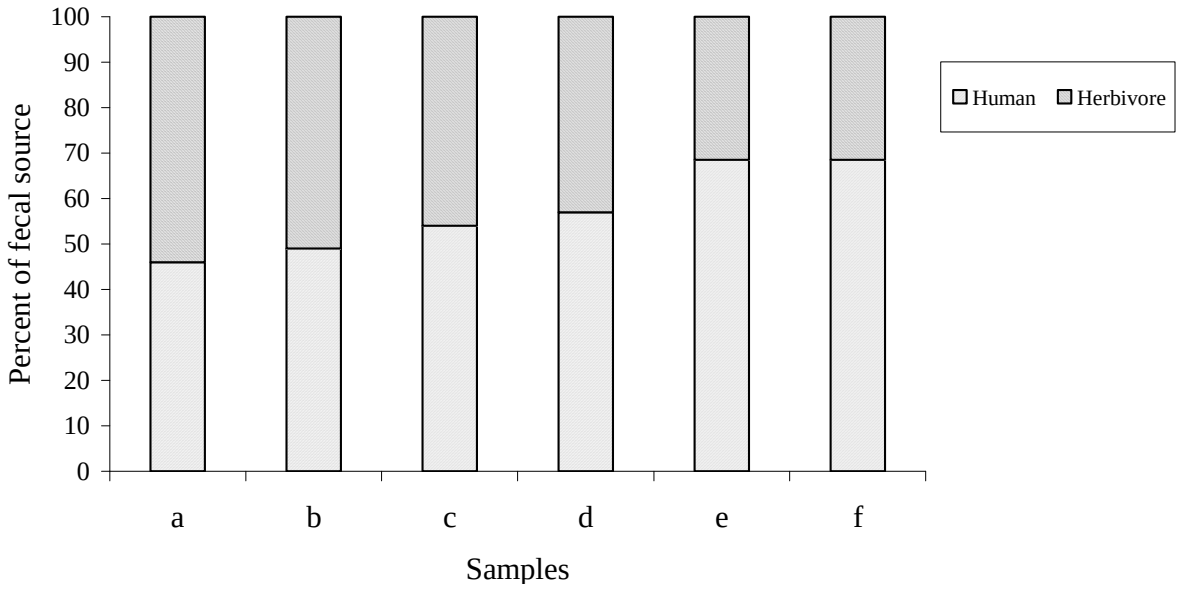


Figure 3.10. Relative contribution (% total) of human and herbivore fecal sources to sediments from Wadi Gaza. Samples a and b were collected in January, 2000 from the west and from the east, respectively. Samples c-f were collected in August, 2001, from the west.

The Wadi Gaza sediment data indicate that between January 2000 and August 2001 most sediments showed an increase in the concentration of coprostanol (Table 3.6). The areas of highest concentration were located adjacent to the coast of the Mediterranean Sea and were influenced by untreated waste water discharge. The mean concentration of coprostanol was generally higher in sediments collected during the later sampling (2001). This observation is consistent with an increasing discharge of sewage to the western part of the Wadi Gaza after 2000.

3.10 Cyanobacterial mats

Most cyanobacteria contain small amounts of short-chain hydrocarbons in the range of C_{15}-C_{21}, often with either *n*-C_{17} or *n*-$C_{17:1}$ predominating (Han et al., 1968a; Han and Calvin, 1969; Winters et al., 1969; Gelpi et al., 1970; Paoletti et al., 1976; Murata and Nishida, 1987; Grimalt et al., 1992). The extractable lipid fraction of natural cyanobacterial mats from Wadi Gaza contains hydrocarbon and fatty acid components that are a combination of biologically synthesized lipids and diagenetically modified materials. Anthropogenically derived materials, primarily hydrocarbons, which result from petroleum use, also contribute to the extracted lipids. Figure 3.11 total ion current, shows the chromatogram of the extractable lipid fraction and the corresponding mass chromatogram of *m/z* 71 for the distribution pattern of the *n*-alkanes. The distribution of the *n*-alkanes in the lipid fraction ranges from 12 to 27 carbon atoms. The *n*-alkane distribution pattern exhibits a maximum at C_{17} and smooth distributions of odd-and even-carbon-numbered *n*-alkane homologues (Figure 3.10). The contribution of the cyanobacteria is reflected in a high CPI_{17} value (CPI ($2C_{17}/[C_{16}+C_{18}]$) of 1.8. This high content of the *n*-C_{17} alkane in the cyanobacterial mats shows a strong evidence that the high content of the *n*-C_{17} alkane in Wadi Gaza sediments is due to the contribution of cyanobacteria. As expected, the signal of higher terrestrial plants is very low in the cyanobacterial mats sample. Diesel oil or other petroleum derivatives are responsible for the rest of the *n*-alkane pattern in the lipid fraction. The same is true for the presence of fossil fuel biomarkers, e.g. pristane and phytane.

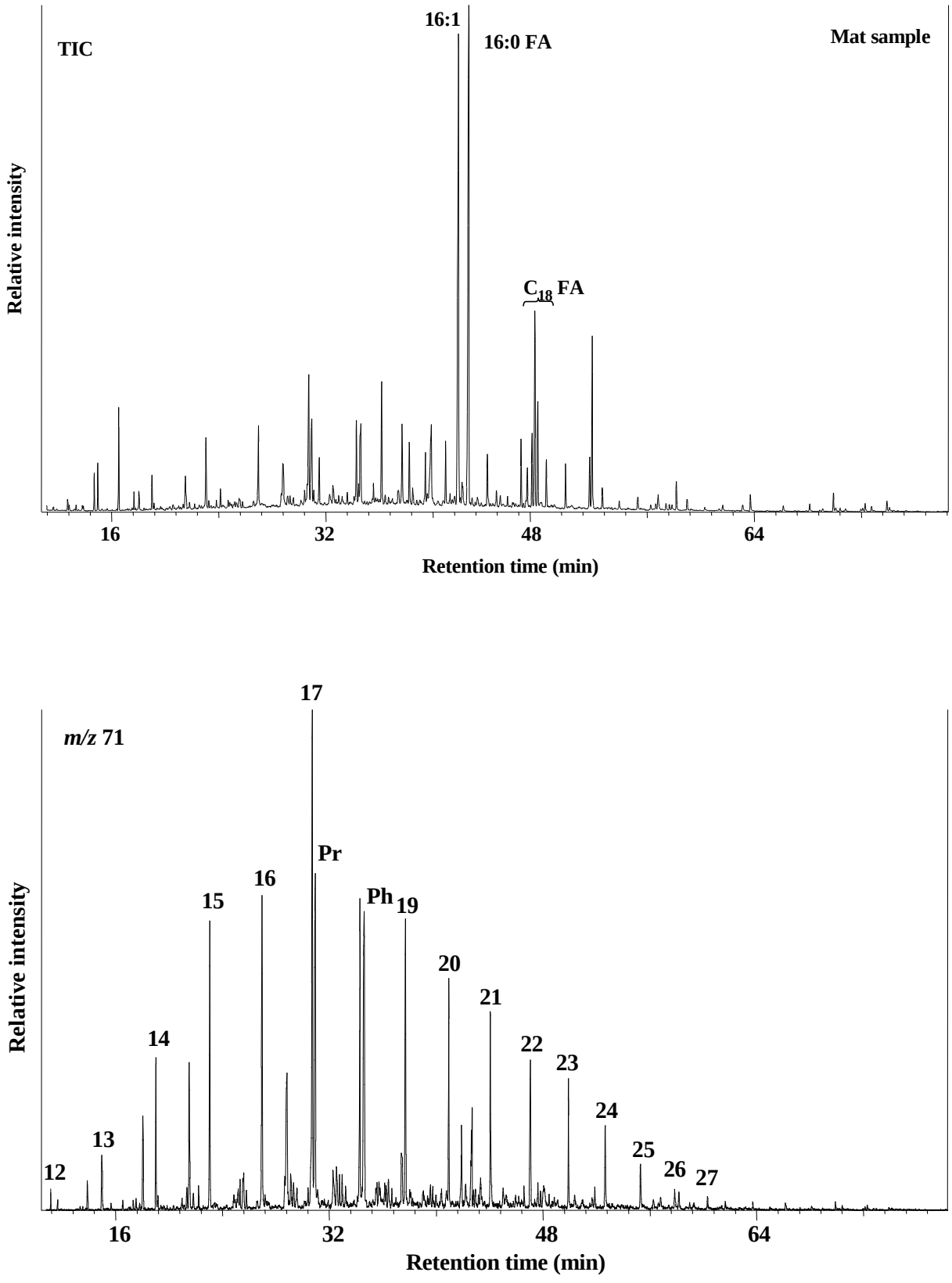


Figure 3.11. Ion chromatograms from GC-MS analysis of a moderately contaminated cyanobacterial mat (total extract, western part of Wadi Gaza); top: reconstructed total ion current chromatogram; bottom: mass chromatogram of *m/z* 71 showing the *n*-alkane distribution. FA: fatty acids, Pr: pristane, Ph: phytane, carbon numbers of *n*-alkane are indicated.

3.11 Conclusions

This study documents the first comprehensive organic geochemical analysis of surficial sediments from Wadi Gaza. The organic matter composition of extractable lipids in the sediments indicates that the Wadi Gaza receives a moderate supply of anthropogenic material. By using GC and GC/MS, aliphatic hydrocarbons from petroleum sources were identified in the Wadi Gaza sediments. The petroleum origin is indicated by the aliphatic isoprenoid alkanes (pristane and phytane), the presence of an unresolved complex mixture, the UCM/NA ratio, the low carbon preference index (CPI ca. 1) and the presence of petroleum biomarkers such as steranes and hopanes. The biomarker parameters based on hopanes and steranes reveal significant differences between the sediment samples which suggest that there are multiple sources of oil or refinery products. Sample d shows strong evidence that microbial degradation of petroleum hydrocarbons has already started. The higher abundance of phytane, compared to the C_{18} *n*-alkane in samples a, d and f is due to the degradation of the *n*-C_{18} alkane mainly by cyanobacterial mats.

In the aromatic hydrocarbon fraction the petroleum origin was indicated by the presence of phenanthrene and its alkylated homologues as well as that of dibenzothiophene and its alkylated homologues. The ratio of methylphenanthrenes to phenanthrene shows that the sediments are dominated by fossil fuel derived phenanthrenes. The methyldibenzothiophene index (MDI) of Wadi Gaza sediments confirms the input of crude oil and petroleum derivatives. The presence of free sterathiols in the sediment can be taken as an indicator for bacterial sulfate reduction after marine incursions.

Compounds deriving from terrestrial sources (higher plants), such as long-chain *n*-alkanes, *n*-fatty acids and *n*-alcohols, and marine-derived hydrocarbons were also present. The high content of the *n*-C_{17} alkane in the cyanobacterial mats shows a strong evidence that the high content of this *n*-alkane in Wadi Gaza sediments is due to the contribution from the cyanobacteria.

The presence of coprostanol and ethylcoprostanol in all Wadi Gaza sediments indicates that the sediments are contaminated with fecals from humans and herbivores. Information from this study should be useful in designing future strategies for environmental protection and management of the wetland of Wadi Gaza.

3.12 Degradation of Egyptian crude oil immobilized on clay by cyanobacterial mats

3.12.1 Characterization of the stabilized Egyptian crude oil

The gas chromatogram of the crude oil (see Appendix, Figure A1) indicates that the dominant hydrocarbons are normal alkanes (C_{10-40}) with the higher molecular weight species (i.e. $>C_{10}$) maximizing at *n*-C_{15} and exhibiting little or no odd-over-even carbon number predominance. The amount of C_{14} to C_{35} *n*-alkanes represent 69.3 µg/mg oil.

3.12.2 Degradation of n-alkanes

In the bioremediation experiment with the oil-loaded organo-clay complex in a slurry with Wadi Gaza cyanobacterial mats, 16.67 mg of the stabilized oil was added to the flasks. *n*-Alkanes from C_{14} to C_{35} were present at the start of the experiment. The C_{10-13} *n*-alkanes were lost during loading of the hydrophobic clay due to the high volatility of these compounds. Extraction of the suspension at day zero yielded 80% of the theoretical amount of the crude oil. This level of recovery is commonly obtained as a result of the experimental conditions, i.e. irreversible adsorption of alkanes to the clay.

Degradation of the C_{14} to C_{35} *n*-alkanes of the loaded oil was studied by their quantification and disappearance in the course of the experiment (Table 3.7 and Figure 3.12). The concentrations of extractable C_{14} to C_{35} *n*-alkanes decreased continuously with time. In the control flask (Table 3.7 and Figure 3.13A-D) there was little or no change in the concentration of the residual total extractable *n*-alkanes during the time of 0 to 60 days. The preferential removal of C_{14}-C_{35} *n*-alkanes relative to branched and cyclic alkanes is now universally recognized as a standard feature in assessing biodegradation of crude oils. This was documented for the first time by Winters and Williams (1969) in their study of the Bell Creek field in the Powder Basin (U.S.A.). Several other examples are listed in books and review papers by Tissot and Welte (1978), Hunta (1979) and Milner et al. (1977).

The analysis of all *n*-alkanes from C_{14} to C_{35} of the crude oil in the course of the experiment indicates that 51% and 56% were degraded after fifteen days under light and dark conditions, respectively. The degradation increased to 63% at day 40 and to 79% at day 60 in the light. In the dark, it remained nearly constant until day 40 and increased to 68% at day 60 (Table 3.7 and Figure 3.13D). A slight decrease was observed in the control in the course of the experiment after 60 days.

The data show that the biodegradation rates of *n*-alkanes can be categorized into three groups: 1) short-chain *n*-alkanes from C_{14} to C_{20} were degraded most rapidly, 2) medium-chain-length *n*-C_{21} to *n*-C_{27} alkanes were degraded the slowest and 3) long-chain *n*-C_{28} to C_{35} alkanes were degraded at intermediate rates.

After fifteen days treatment in the light, 51% of the short chain length *n*-C_{14} to C_{20} were degraded. In the dark, 55% of this group had disappeared (Table 3.7 and Figure 3.13A).

Table 3.7. Quantities (µg) of residual *n*-alkanes of Egyptian crude oil during biodegradation by cyanobacterial mats from Wadi Gaza (slurry experiments).

Time Days	Light				Dark				Control			
	C_{14-20}	C_{21-27}	C_{28-35}	C_{14-35}	C_{14-20}	C_{21-27}	C_{28-35}	C_{14-35}	C_{14-20}	C_{21-27}	C_{28-35}	C_{14-35}
0	8.6	6.8	3.4	18.7	7.2	5.7	2.9	15.8	7.4	7.3	3.8	18.5
15	4.2	3.3	1.6	9.1	3.2	2.6	1.1	6.9	8.4	7.6	3.8	19.8
30	3.6	3.4	1.4	8.4	3.1	2.7	0.9	6.7	8.1	7.8	4.0	19.9
40	2.8	3.0	1.1	6.9	3.4	2.9	1.0	7.3	7.0	7.7	4.0	18.7
50	1.3	2.3	0.8	4.4	1.7	2.6	0.9	5.2	6.6	6.8	3.3	16.7
60	1.1	2.2	0.7	4.0	1.6	2.6	0.8	5.0	6.6	7.6	4.0	17.1

Light = 12 h light/12 h darkness; Dark = 24 h darkness; Control = 12 h light/12 h darkness (no cyanobacterial mat).

In the samples of day 40, the degradation of this group had increased to 67% in the light and remained nearly constant in the dark. At the end of the experiment after 60 days, 87% and 78% of this group had been degraded in the light and dark, respectively. A lower recovery and thus a slight decrease of the short chain length *n*-C_{14} to C_{20} alkanes relative to the other groups was also found in the control in the time period of 40-60 days (Table 3.7 and Figure 3.13A). The higher degradation rates of the *n*-alkanes in group 1 may be due to their low molecular weight and their greater accessibility to the bacteria, i.e. ease of transport through the cell wall (Walker and Colwell 1976; Stafford et al., 1982).

In the case of the second group with medium-chain-length alkanes from *n*-C_{21} to *n*-C_{27}, 51% and 54% were degraded within the first fifteen days in the light and the dark, respectively. Degradation of this group of alkanes increased to 56% and 67% in the light at 40 and 60 days, respectively. The degradation remained nearly constant in the dark until the end of the experiment. In the control, there is no decrease in this group in the course of the experiment except for the sample at day 50 (Table 3.7 and Figure 3.13B) which is considered

an analytical artifact considering the higher value at 60 days. These results are probably linked to the reduced ability of the bacteria to attack the medium chain-length *n*-alkanes (Badawy and Al-Harthy, 1991; Badawy et al., 1993).

In the third group with long chain *n*-alkanes with 28 to 35 carbon atoms, 53% and 63% were degraded after fifteen days in the light and dark, respectively. The degradation of this group increased to 68% at day 40 and to 80% at day 60 in light whereas it remained nearly constant in the dark until day 40 and increased to 77% at day 60 (Table 3.7 and Figure 3.13C). No decrease was observed for this group in the control in the course of the experiment except for the sample at day 50, considered an analytical artifact.

Biodegradation of the *n*-alkanes in the present study is considered to be moderate, since after 60 days still a residue of 21% and 32% was unaffected under light and dark conditions, respectively. A comparison of the microbial degradation in this experiment with literature data is possible only to a limited extent since there are important differences between culture approaches, e.g. the use of intact mats and slurry experiments or the use of organically modified clays which is essentially unique to this study. *n*-C_{12}-C_{26} alkanes of an Egyptian crude oil were degraded by a mixed culture of bacteria within one month as reported by El-Rafie et al. (2001). Mixed cultures of the marine cyanobacterial species *Oscillatoria salina*, *Plectonema terebrans* and *Aphanocapsa sp*. removed 42% of a Bombay High crude oil (containing 50% aliphatic hydrocarbons) in 10 days. In addition, 65% of pure *n*-hexadecane (C_{16}) as a model compound were degraded by *Aphanocapsa sp*. within 10 days (Raghukumar et al., 2001). *Acinetobacter* sp. A3 isolated from crude oil refinery sludge was able to degrade 60% of the same Bombay High crude oil after 5 days of incubation in shake flask experiments, and the bacteria utilized the oil as their sole source of carbon (Hanson et al., 1997). Palittapongarnpim et al. (1998) reported degradation of 40% of *n*-alkanes of Tapis crude oil within 7 days by bacteria. *Klebsiella* sp. KCL-1 isolated from seawater used *n*-alkanes from *n*-octadecane (C_{18}) to *n*-hexacosane (C_{26}) of a crude oil as their sole carbon source (Cha et al., 1999). Most of the *n*-alkanes in Kuwait crude oil were degraded by *Pseudomonas* strains as reported by Al-Gounaim et al. (1995).

A strain of *Yarrowia lipolytica* isolated from a chronically oil-polluted tropical marine estuary degraded 78% and 92% of the aliphatic fraction of Bombay High crude oil in free and immobilized form, respectively (Zinjarde et al., 2000). The *Rhodococcus sp*. isolated from a chronically oil-polluted marine site degraded 50% of the aliphatic fraction of Assam crude oil after 72 h incubation as reported by Sharma and Pant (2000). In addition, Whyte et al. (1998) and Milekhina et al. (1998) reported degradation of alkanes up to *n*-C_{30}

by various strains of *Rhodococcus.* Amin et al. (1995) reported that short- and medium-chain-length hydrocarbons in Egyptian crude oil were readily degraded by mixed microbial populations isolated from soil samples, collected from different sites of oil spills whereas the long-chain hydrocarbons were resistant to biodegradation. A microbial consortium called SM8, isolated from a sediment in Shizugawa Bay (Japan), degraded 50 to 60% of the saturated hydrocarbon fraction of a crude oil in 30 days in batch culture (Ishihara et al., 1995). During aerobic biodegradation of a crude oil carried out in the laboratory, Pond et al. (2001) found that C_{14} to C_{28} *n*-alkanes were degraded and that the C_{14} to C_{18} homologues were degraded most rapidly. This is in agreement with the faster degradation of the first group of *n*-alkanes observed in our experiment.

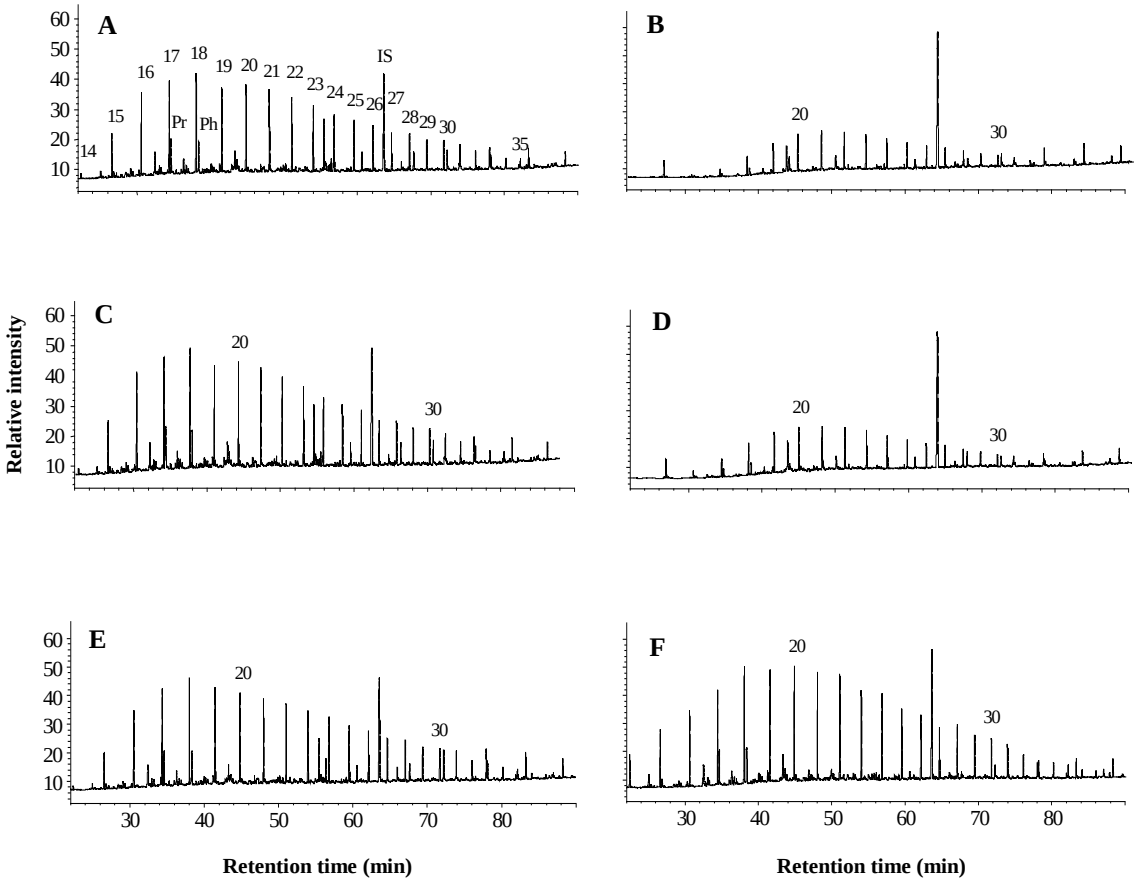


Figure 3.12. Representative gas chromatograms of extracts from oil-loaded organo-clay complexes in a slurry experiment with Wadi Gaza cyanobacterial mats. A and B: 12 h light/12 h darkness; C and D: 24 h darkness; E and F: controls without mats 12 h light/12 h darkness. Samples A, C and E were taken at time zero; samples B, D and F were taken after 60 days. Numbers indicate carbon numbers of *n*-alkanes. IS: internal standard; Pr: pristane; Ph: phytane.

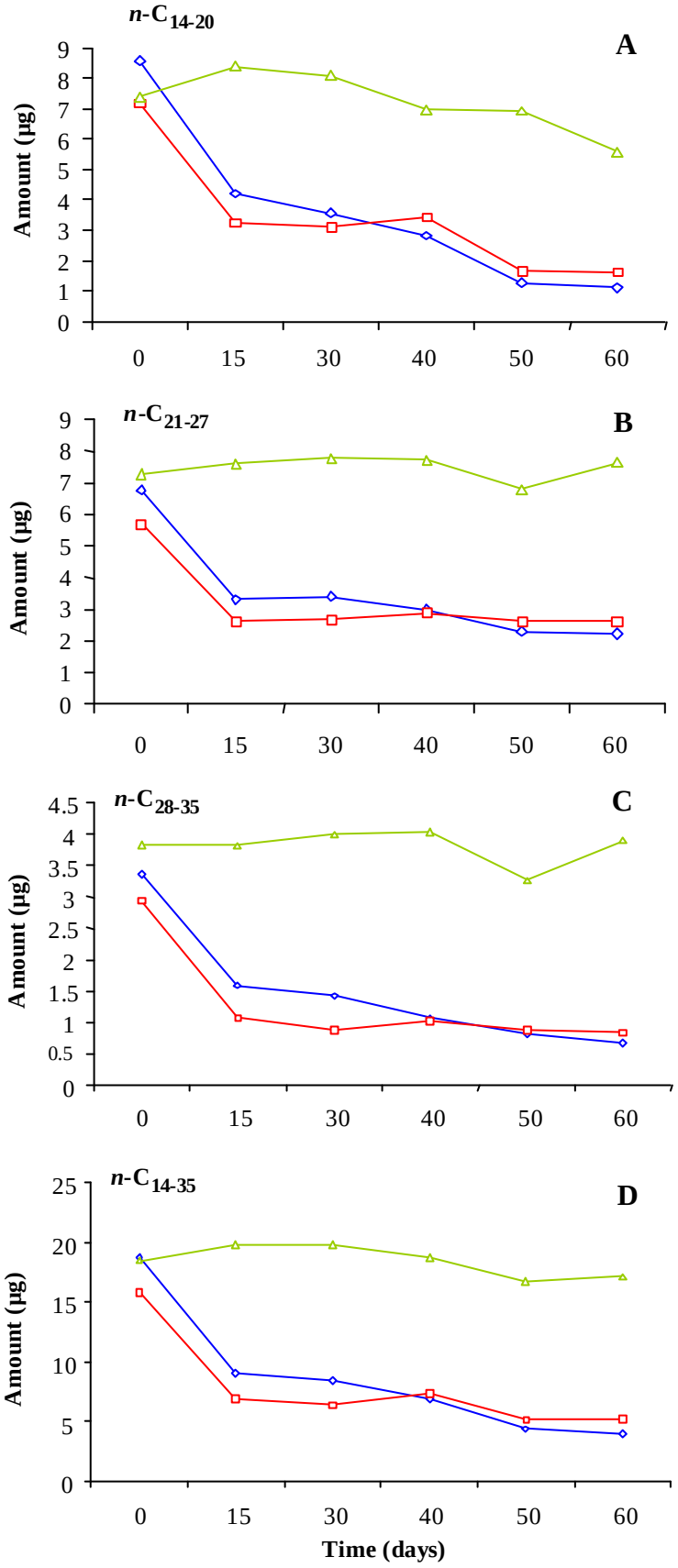


Figure 3.13. Quantities of residual *n*-alkanes in crude oil-loaded organo-clay complexes in a slurry experiment with Wadi Gaza cyanobacterial mats vs. time: 12 h light/12 h darkness 24 h darkness control 12 h light/12 h darkness.

Medium-and long-chain *n*-alkanes with up to 40 carbon atoms served as sole carbon and energy source for *Acinetobacter calcoaceticus* at the Arabian Gulf coast polluted with oil (Radwan et al., 1999). A phenanthrene-utilizing bacterium (strain AR-3) degraded 50% of the saturated hydrocarbon fraction of a Malaysian crude oil within ten days (Law and Teo, 1997). The biodegradation percentage of normal and branched alkanes of Alwyn crude oil ranged from 75% to 85% in an immobilized system, whereas 60-66% were biodegraded in the free suspension system with the halotolerant bacterial consortium MPD-M after an incubation period of 20 days (Díaz et al., 2002).

The biodegradation rates for *n*-alkanes in crude oils tend to decrease as chain length increases (Holder et al., 1999; Pond et al., 2001). In our experiment we found that the long-chain *n*-alkanes from C_{28} to C_{35} were degraded faster than those of medium chain length with 21 to 27 carbon atom. This suggests that the degradation pattern is due to the specific composition of the microbial community. Natural microbial populations in seawater biodegraded 28% of crude oil within 8 weeks as reported by Dutta et al. (2000). Since in our experiment 80% of the *n*-alkanes of the crude oil were degraded within 60 days, we conclude that the degradation rates of the *n*-alkanes in our experiment was fast compared to other experiments reported in the literature.

According to GC-MS analysis no peaks of new substances appear in the chromatograms. This indicates that no accumulation of possible metabolites or degradation products could be observed in this experiment. This suggests that degradation of the crude oil components has led to complete mineralization. This may be favored by the presence of a complex microbial community in the cyanobacterial mat material. The Office of Technology Assessment (1991) reported that no single species of microorganism can degrade all the components of a given oil. Effective bioremediation of petroleum contamination requires a mixture of populations consisting of different genera each capable of metabolizing different types of compounds (Dave et al., 1994; Radwan et al.,1995; Korda et al., 1997). The degradation rate decreased after the first two weeks under both light and dark conditions. This may indicate that the crude oil had a toxic effect on the growth of certain microorganisms. Many studies have demonstrated that crude oil contains constituents which are inhibitory to microorganisms even at low concentrations (Radwan and Al-Hasan, 2000 and references therein).

3.12.3 The pristane/n-C_{17} and phytane/n-C_{18} ratios

The pristane to *n*-heptadecane (C_{17}) and phytane to *n*-octadecane (C_{18}) ratios were measured to provide an estimation of the degree of biodegradation of *n*-alkanes relative to branched compounds in the saturated hydrocarbon fraction. Changes in relative abundance of the C_{19} and C_{20} isoprenoid alkanes, pristane and phytane, to their associated *n*-C_{17} and *n*-C_{18} alkanes, heptadecane and octadecane, have often been used to assess the degree of microbial degradation that an oil has undergone (Jobson et al., 1972; Bailey et al., 1973a; Westlake et al., 1974; Pritchard and Costa, 1991; and Volkman et al., 1997, and references therein).

Table 3.8. Ratios of pristane/*n*-C_{17} and phytane/*n*-C_{18} in an Egyptian crude oil degraded by Wadi Gaza cyanobacterial mats.

Time Days	Light		Dark		Control	
	Pr/*n*-C_{17}	**Ph/*n*-C_{18}**	**Pr/*n*-C_{17}**	**Ph/*n*-C_{18}**	**Pr/*n*-C_{17}**	**Ph/*n*-C_{18}**
0	0.53	0.52	0.53	0.52	0.53	0.52
15	0.93	0.95	0.84	0.85	0.52	0.51
30	1.08	1.04	0.84	0.76	0.52	0.51
40	1.00	0.78	0.80	0.71	0.53	0.50
50	0.71	0.66	0.78	0.75	0.52	0.51
60	0.60	0.58	0.71	0.58	0.53	0.51

Light = 12 h light/12 h darkness; Dark = 24 h darkness; Control = 12 h light/12 h darkness.

Bacteria readily degrade normal alkanes whereas the isoprenoid alkanes are relatively resistant to microbial degradation, resulting in an increase in the Pr/*n*-C_{17} and Ph/*n*-C_{18} ratios with increasing level of degradation. Many authors have used this observation to assess the degree of oil degradation in natural systems and following bioremediation (Atlas et al., 1981; Basseres and Ladousse, 1993). Changes in pristane to *n*-heptadecane (C_{17}) and phytane to *n*-octadecane (C_{18}) ratios for light, dark and control samples are presented in Table 3.8 and Figure 3.14. In the course of the experiments, the ratios increased when degradation occurred in both light and dark samples. The pristane/*n*-C_{17} and phytane/*n*-C_{18} ratios increased from about 0.5 to 1.08 and 1.04 in the light and 0.84 and 0.76 in the dark, respectively, within the first 30 days. In the control sample, the ratios are nearly similar to each other and were constant over the course of the experiment (Table 3.8 and Figure 3.14). Surprisingly, the ratios decreased again in the period of 30 to 60 days under both light and dark conditions.

This is due to faster degradation of the isoprenoid alkanes after 40 days. This suggests that adaptation occurs in the cyanobacterial mat organisms during the experiment.

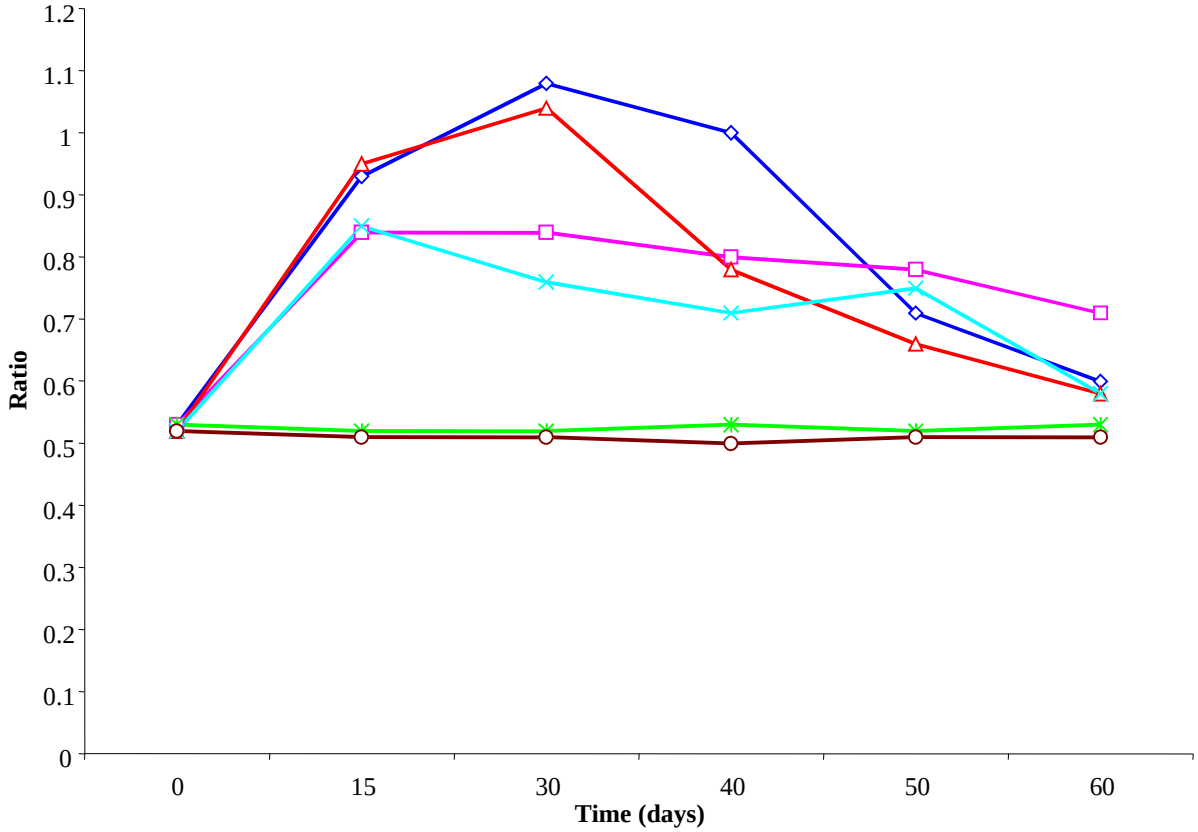


Figure 3.14. Pristane/*n*-C_{17} and phytane/*n*-C_{18} ratios in crude oil degraded by cyanobacterial mats from Wadi Gaza in a slurry experiment with crude oil-loaded organo-clay complexes, pristane/*n*-C_{17}: 12 h light/12 h dark, pristane/*n*-C_{17}: 24 h darkness, phytane/*n*-C_{18}: 12 h light/12 h dark, phytane/*n*-C_{18}: 24 h darkness, pristane/*n*-C_{17}: Control, 12 h light/12 h, phytane/*n*-C_{18}: control, 12 h light/12 h dark.

3.12.4 Degradation of n-heptadecane (C_{17}) and n-octadecane (C_{18})

As indicated in Figure 3.15 and Table 3.9, 53% and 56% of the *n*-heptadecane (C_{17}) were degraded in the light and in the dark, respectively, within the first two weeks. At the end of the experiment 95% of the *n*-heptadecane (C_{17}) was degraded in the light and 87% in the dark. The same degradation percentage, i.e. 53% and 56%, was observed for *n*-octadecane (C_{18}), in the light and in the dark, respectively, within the first two weeks. 88% and 75% of *n*-octadecane (C_{18}) were degraded in the light and dark, respectively, at the end of the experiment. No significant decrease was observed for *n*-heptadecane (C_{17}) and *n*-octadecane (C_{18}) in the control sample (Table 3.9). In general, biodegradation of *n*-C_{17} was slightly more advanced than that of *n*-C_{18} in both light and dark experiments after two weeks.

A remaining concentration of *n*-octadecane (C_{18}) at a level of about 20% was already observed in all earlier slurry and mesocosm degradation experiments with *n*-C_{18} as model compound on organo-clay complexes (Abed et al., 2002 ; Safi et al., 2003). Sixty percent, and 50% of pure *n*-C_{17} and *n*-C_{18} alkanes, respectively, were degraded by *Pseudomonas aeruginosa* in the presence of molasses whereas 22% of *n*-C_{18} were degraded in the presence of a mineral fertilizer in 24 h (Al-Hadhrami et al., 1997).

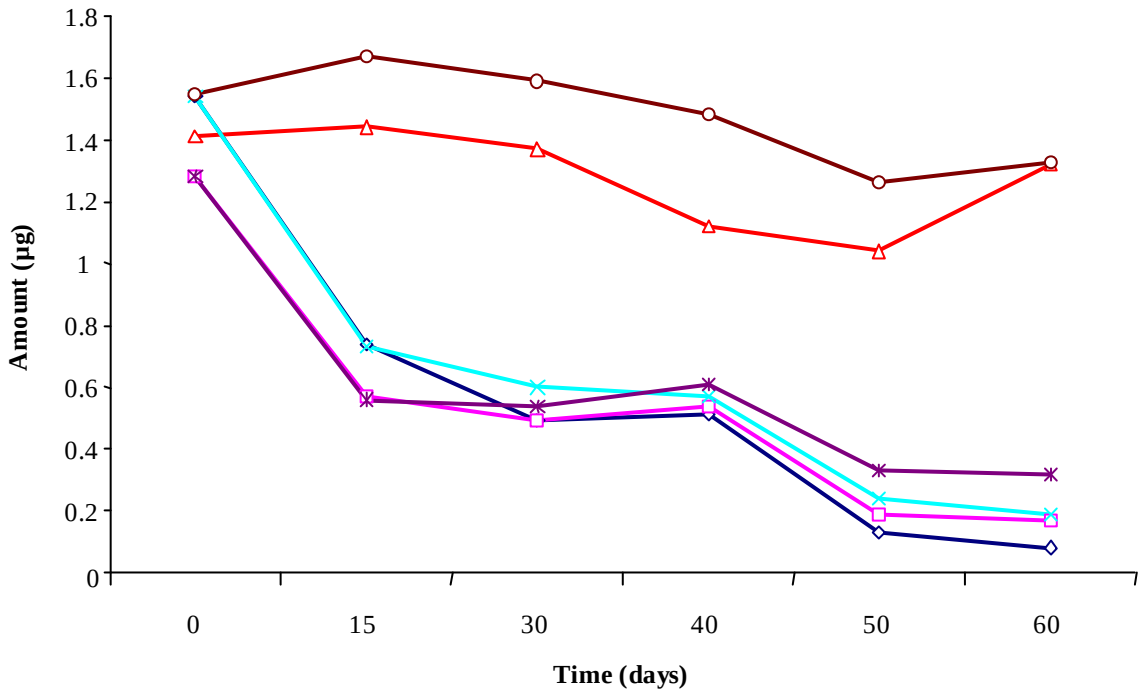


Figure 3.15. Biodegradation of C_{17} and C_{18} *n*-alkanes in crude oil-loaded organo-clay complexes in a slurry experiment with cyanobacterial mats from Wadi Gaza, *n*-C_{17} 12 h light/12 h dark, *n*-C_{17} 24 h darkness, *n*-C_{17} control, 12 h light/12 h dark, *n*-C_{18} 12 h light/12 h dark, *n*-C_{18} 24 h darkness, control, 12 h light/12 h dark.

n-Heptadecane (C_{17}) and *n*-octadecane (C_{18}) were completely degraded in immobilized cells whereas 78-91% were degraded in a free suspension system with the halotolerant bacterial consortium MPD-M after 20 days of incubation (Díaz et al., 2002). Radwan et al. (1999) showed that 74% of pure *n*-octadecane (C_{18}) were degraded by *Acinetobacter calcoaceticus* within 7 days. Grötzschel et al. (2002) reported that 60% of *n*-octadecane (C_{18}) were degraded in intact cyanobacterial mat experiments with organo-clay complexes within four months. In the present experiment, a similar extent of *n*-heptadecane (C_{17}) and *n*-octadecane (C_{18}) degradation of 95% and 88%, respectively, in 60 days was

determined (Table 3.9). We therefore conclude that the degradation rates of theses *n*-alkanes were high.

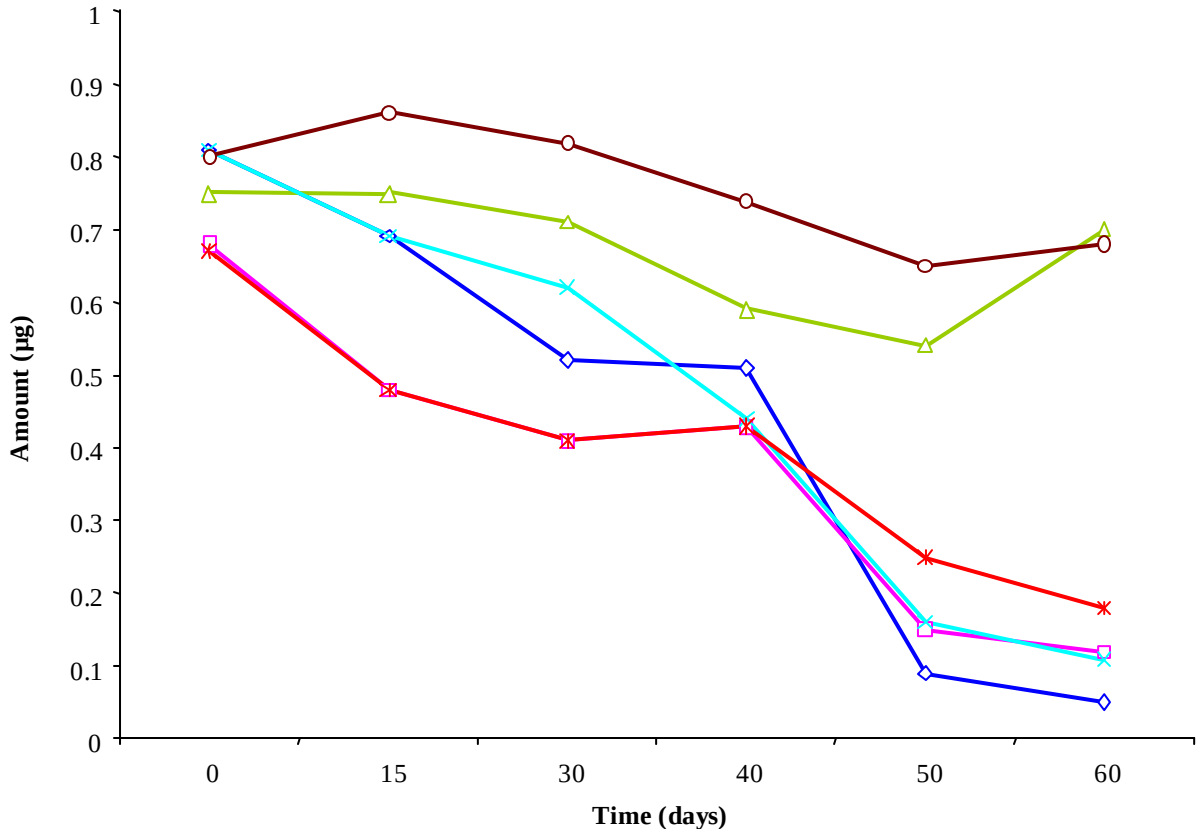


Figure 3.16. Biodegradation of pristane and phytane in crude oil-loaded organo-clay complexes in a slurry experiment with cyanobacterial mats from Wadi Gaza pristane:12 h light/12 h dark, pristane: 24 h darkness, pristane: control, 12 h light/12 h dark, phytane: 12 h light/12 h dark, phytane: 24 h darkness, phytane: control, 12 h light/12 h dark.

3.12.5 Degradation of pristane and phytane

Figure 3.16 presents the results of the slurry experiments regarding the biodegradation of the C_{19} and C_{20} isoprenoid alkanes, pristane and phytane, under both light and dark conditions. The data show that the cyanobacterial mats were able to degrade these compounds. At day 40, 37% of the pristane was degraded in the light and in the dark. At the end of the experiment, 94% of the pristane were degraded in the light and 82% in the dark. The phytane biodegradation pattern was similar to that described for pristane in the course of the experiment. In the light, 63% of phytane were degraded. The degradation of phytane increased to 75% and 88% in the dark and light, respectively (Table 3.9). An apparently non-degradable residual concentration of pristane at the 20% level was already observed in all earlier degradation experiments with organo-clay complexes (Abed et al., 2002; Safi et al., 2003).

Table 3.9. Quantities (µg) of residual *n*-C_{17}, *n*-C_{18} alkanes, pristane and phytane in an Egyptian crude oil after biodegradation by cyanobacterial mats from Wadi Gaza.

Time Days	Light				Dark				Control			
	***n*-C_{17}**	***n*-C_{18}**	**Pr**	**Ph**	***n*-C_{17}**	***n*-C_{18}**	**Pr**	**Ph**	***n*-C_{17}**	***n*-C_{18}**	**Pr**	**Ph**
0	1.5	1.5	0.8	0.8	1.3	1.3	0.7	0.7	1.4	1.6	0.8	0.8
15	0.7	0.7	0.7	0.7	0.6	0.6	0.5	0.5	1.4	1.7	0.8	0.9
30	0.5	0.6	0.5	0.6	0.5	0.5	0.4	0.4	1.4	1.6	0.7	0.8
40	0.5	0.6	0.5	0.4	0.5	0.6	0.4	0.4	1.1	1.5	0.6	0.7
50	0.1	0.2	0.1	0.2	0.2	0.3	0.2	0.3	1.0	1.3	0.6	0.7
60	0.1	0.2	0.05	0.1	0.2	0.3	0.1	0.2	1.3	1.3	0.7	0.7

Light = 12 h light/12 h darkness; Dark = 24 h darkness; Control = 12 h light and 12 h darkness; Pr = pristane; Ph = phytane.

Pristane and phytane are widely used as largely recalcitrant biomarkers in geochemistry, and their bioremediation is strongly related to the degradation conditions: 10 g/l of pristane were degraded by a pure culture of *Rhodococcus equi* P1 in 24 h (Ko and Lebeault, 1999), whereas a 85% degradation of 470 µmol/l pristane in an anaerobic sediment slurry took six months (Grossi et al., 2000). The removal of pristane was 63% in a four month experiment with intact cyanobacterial mats from Solar Lake in Sinai, Egypt (Grötzschel et al., 2002). Grossi et al. (2002) reported 35% of pristane and phytane were degraded in marine coastal sediments after 12 months of in situ incubation. The biodegradation percentages of pristane and phytane were 86% and 94%, respectively, with immobilized cells whereas the extent of degradation was 40% and 25%, respectively, in the free suspension system with the halotolerant bacterial consortium MPD-M in batch cultures over 20 days (Díaz et al., 2002). Nakajima et al. (1985) and Sharma and Pant (2000) reported pristane degradation by various strains of *Rhodococcus.*

Pristane and phytane are more resistant to biodegradation than normal alkanes, as observed by Atlas (1981) and Basseres and Ladousse (1993). Since in the present experiment 94% and 88% of pristane and phytane, respectively, were degraded in 60 days, we conclude that the degradation rates of these isoprenoidal alkanes were high**.**

3.12.6 Degradation kinetics

The terms first and zero order come from chemical kinetics. In a first-order process, the rate is proportional to the concentration of a single substrate. In a zero-order process, the rate is independent of the substrate concentration. When the concentration is plotted against time,

the concentration decreases at a constant rate in zero-order processes, but it falls quickly initially and then more slowly in first-order processes (Alexander, 1999).

From regression analysis of the data for the Egyptian crude oil it is obvious that degradation fits the first-order kinetics as described by Segel (1976). The equation is $\ln C_t = \ln C_0 - Kt$, where C_0 represents the initial concentration, C_t represents the concentration at time t, t represents the time in days and K represents the decay rate constant. The larger the value of K, the faster is the degradation process. First-order rate constants (K), correlation coefficient (r^2) and the time required for 50% degradation of the tested compounds (Dt_{50}) calculated for the *n*-C_{14} to *n*-C_{35} alkanes of the degraded crude oil are presented in Table 3.10.

Table 3.10. First-order degradation rate constant, correlation coefficient and degradation half-life time of *n*-alkanes of an Egyptian crude oil in a slurry experiment with cyanobacterial mats.

***n*-alkanes →**		**C_{14-20}**	**C_{21-27}**	**C_{28-35}**	**C_{14-35}**
Light	K	0.0336	0.0167	0.0251	0.0245
	r^2	0.94	0.85	0.95	0.94
	Dt_{50}	20	26	21	23
Dark	K	0.0226	0.01	0.0172	0.0161
	r^2	0.84	0.51	0.65	0.75
	Dt_{50}	23	28	20	24

Light = 12 h light/12 h darkness; Dark = 24 h darkness; K = degradation rate constant, r^2 = correlation coefficient and Dt_{50} = degradation half-life time.

It can be seen that the degradation patterns follow an exponential curve in all cases (Figures 3.17 and 3.18). Linear regression based on the logarithm of the concentrations (e.g. [K-C_{14-20}]) was performed to detect any differences between experiments and compound groups. Figures 3.17 and 3.18 show that all differences are in the range of the statistical error, this means degradation in all experiments truly follows first-order kinetics.

The correlation coefficient values (r^2) are higher than 0.8 for all groups except for *n*-C_{21-27}, *n*-C_{28-35} and *n*-C_{14-35} in the dark (Table 3.10 and Figures 3.17 and 3.18). The degradation rate constant (K) for the *n*-alkanes calculated from the above equation are higher for the *n*-C_{14-20} homologues than that for the *n*-C_{21-27} and *n*-C_{28-35} compounds (Table 3.10). These data suggest that degradation of the *n*-C_{21-27} and *n*-C_{28-35} alkanes occurs more slowly than that of the *n*-C_{14-20} homologues.

The relatively high degradation rates of the C_{14-20} *n*-alkanes may be due to their low molecular weight and their greater accessibility to the bacteria (Walker and Colwell 1976; Stafford et al., 1982). These results suggest that degradation is a function of desorption of the *n*-alkanes. Comparing the Dt_{50} values (time required for 50% decay of the parent compound) shows a shorter degradation time for the *n*-C_{14-20} alkanes (20 days) in the light whereas the time required for degradation of the *n*-C_{21-27} and *n*-C_{28-35} alkanes is 26 and 21 days, respectively (Table 3.10).

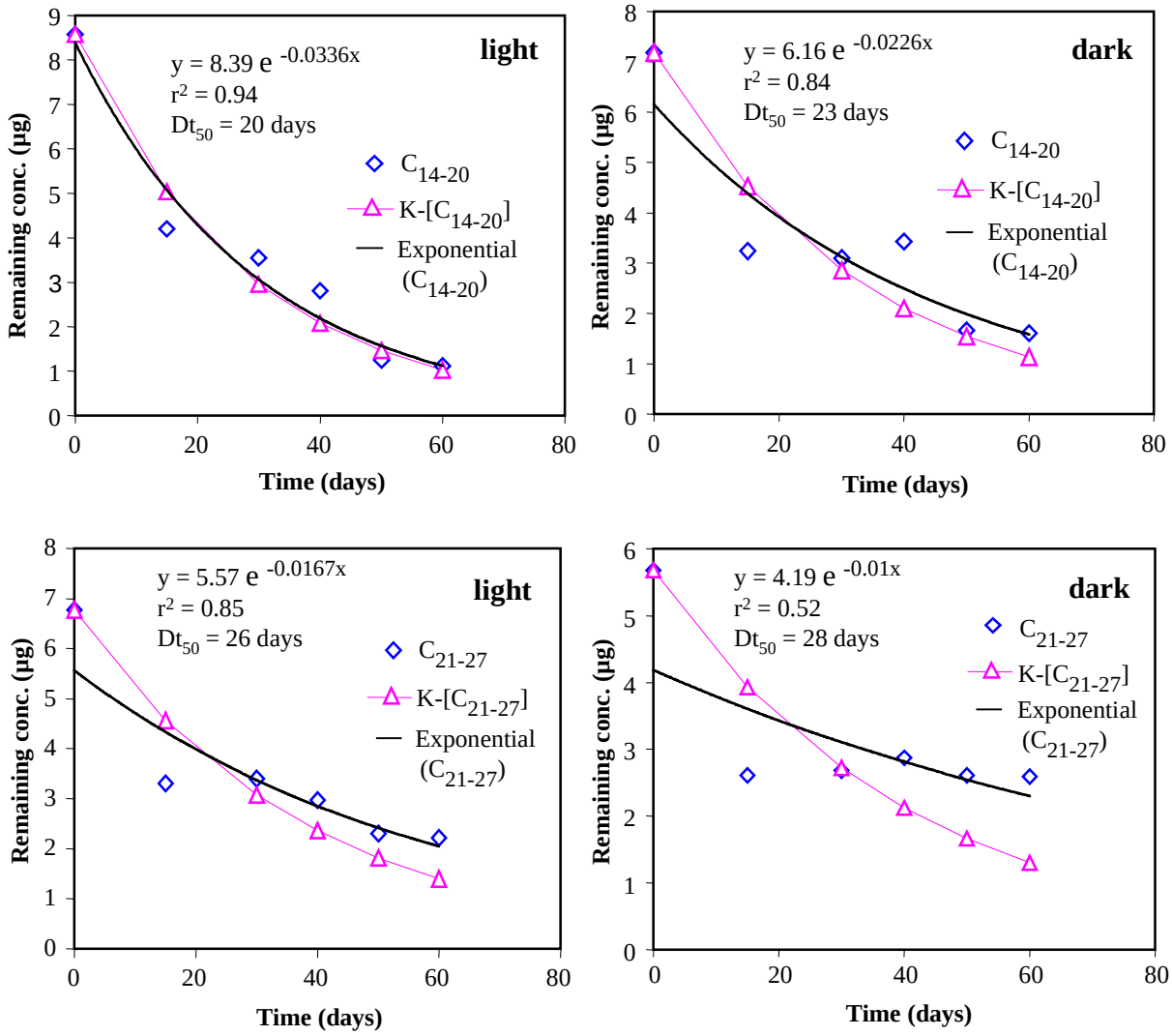


Figure 3.17. Degradation of C_{14-20} and C_{21-27} *n*-alkanes of an Egyptian crude oil in a slurry experiment with Wadi Gaza cyanobacterial mats. Light: 12 h light/12 h darkness; Dark: 24 h darkness ◇ represents experimental data, —△— represents linear regression based on the logarithm of the concentration, r^2: correlation coefficient, and Dt_{50}: degradation half-life time.

In the dark, shorter degradation times are required for *n*-C_{28-35} alkanes (20 days), whereas the time required for degradation of the *n*-C_{14-20} and *n*-C_{21-27} alkanes is 23 and 28 days, respectively. The highest degradation percentages of 87 and 78% were observed for the *n*-C_{14-20} alkanes under light and dark conditions, respectively (Table 3.7). These results suggest that the bio-availability of the *n*-C_{14-20} alkanes is greater than that of the *n*-C_{21-27} and *n*-C_{28-35} alkanes, probably due to adsorption/desorption processes and/or some specific properties of the cyanobacterial mat organisms degrading this group of hydrocarbons. Thus, a characterization of bacterial community and community changes is needed. There are no significant differences in Dt_{50} for *n*-C_{14-35} alkanes between light and dark.

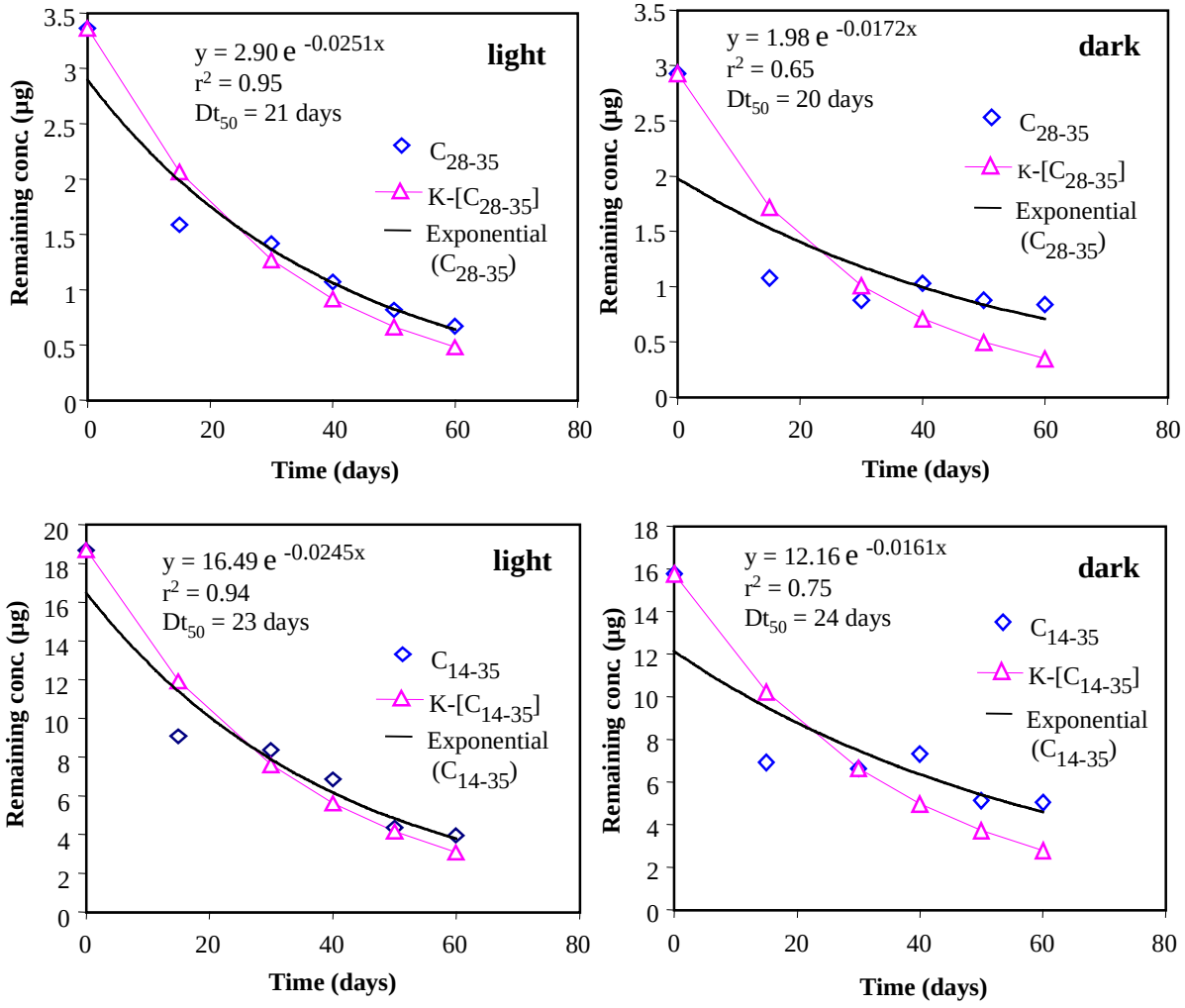


Figure 3.18. Degradation of C_{28-35} and C_{14-35} *n*-alkanes of an Egyptian crude oil in a slurry experiment with Wadi Gaza cyanobacterial mats. Light: 12 h light/12 h darkness; Dark: 24 h darkness ◇ represents experimental data, —△— represents linear regression based on the logarithm of the concentration, r^2: correlation coefficient, and Dt_{50}: degradation half-life time.

3.12.7 Conclusions

This study supports the observation after oil spills in the Arabian Gulf that microbial communities dominated by cyanobacteria can be involved in the degradation of petroleum after pollution of the environment. It also corroborates other previous studies of crude oil degradation by cyanobacteria (e.g. Raghukumar et al., 2001). Natural cyanobacterial mats obtained from chronically oil-polluted environments of Wadi Gaza were able to partially degrade Egyptian crude oil. The mats effectively degraded both *n*-alkanes and the isoprenoids pristane and phytane. The *n*-C_{14-20} alkanes were degraded most rapidly, the *n*-C_{21-27} alkanes were degraded the slowest and the *n*-C_{28-35} alkanes were degraded at intermediate rates. Pristane and phytane were degraded to 94 and 88%, respectively. Organo-clay complexes have been successfully applied as a carrier system for crude oil to the cyanobacterial mat organisms in water and thus allowed the degradation of *n*-alkanes and isoprenoids.

3.13 Degradation of petroleum model compounds by cyanobacterial mats in slurry experiments

Degradation of *n*-octadecane, pristane, phenanthrene, and dibenzothiophene was studied by their disappearance in the course of a series of slurry experiments using cyanobacterial mat material from Wadi Gaza as a source of hydrocarbon-degrading microorganisms (Figure 3.19). The amount of each model compound added to the experimental flasks was 3.33 mg (corresponding to 13.1, 13.9, 18.1 and 18.7 µmol of *n*-octadecane, pristane, phenanthrene and dibenzothiophene, respectively). Extraction of the suspension at day 0 yielded 76 to 84% of the theoretical amount of the four model compounds. This level of recovery is commonly obtained as a result of experimental and analytical conditions. In the control samples with and without dead cyanobacterial mats (Figure 3.19c and d), the amounts and unaltered relative composition of the model compounds indicate that no degradation had occurred. Slightly lower yields were obtained in the control with dead cyanobacterial mats (Figure 3.19c). A lower recovery and a slight decrease of *n*-octadecane relative to the other model compounds was also found in the light control without cyanobacterial mats at day 11; this pattern remained unaltered until 40 days. The concentrations of the four petroleum model compounds applied to the mats decreased steadily (Table 3.11).

Table 3.11. Quantities (µg) of residual model compounds in slurry experiments with cyanobacterial mats from Wadi Gaza.

Time days	Light				Dark			
	Pr	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**
0	49.4	48.2	59.0	51.2	54.3	50.7	62.0	56.1
3	22.6	3.4	1.6	16.9	25.5	13.3	7.6	18.5
7	20.9	0.0	0.1	16.8	20.9	0.0	0.0	15.7
11	18.3	0.0	0.0	14.5	19.3	0.0	0.0	15.3
	Control[1)]				**Control[2)]**			
	Pr	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**
0	48.2	47.0	57.2	49.7	58.7	55.3	64.5	60.9
3	44.4	39.1	46.2	47.4	55.8	48.5	56.3	58.5
7	35.4	30.8	34.2	39.6	53.3	47.8	49.1	57.3
11	48.2	38.0	37.5	53.1	58.1	54.0	63.1	60.9

Pr: pristane; DBT: dibenzothiophene; Phen: phenanthrene and *n*-C_{18}: *n*-octadecane; Light: 12 h light/12 h darkness, Dark: 24 h darkness; 1) control with autoclaved mats and 2) control without mats.

The aromatic substances phenanthrene and dibenzothiophene were degraded faster than the aliphatic compounds pristane and *n*-octadecane. Already after three days treatment in the light, 93% and 98% of dibenzothiophene and phenanthrene, respectively, were degraded (Figure 3.19a). In the dark, 74 and 89%, respectively, of these two aromatic compounds were removed (Figure 3.19b). In the samples of day 7 and later, these compounds were completely degraded both in the light and in the dark. After three days, degradation of the aliphatic compounds pristane and *n*-octadecane had also started. In the case of *n*-octadecane, the remaining amount after three days was ca. 32% both in the light and in the dark. Similarly, pristane was degraded to 42% in the light and to 38% in the dark (Figures 3.19a and b). During the rest of the experiment until day 40, the amounts of pristane and *n*-octadecane remained constant at 36 and 27%, respectively. The decreasing concentrations of the petroleum model compounds (Table 3.11), as well as the changes in their relative amounts, indicated a high biodegradation efficiency in the course of the experiment. Major loss due to photooxidation, which might account for the disappearance of

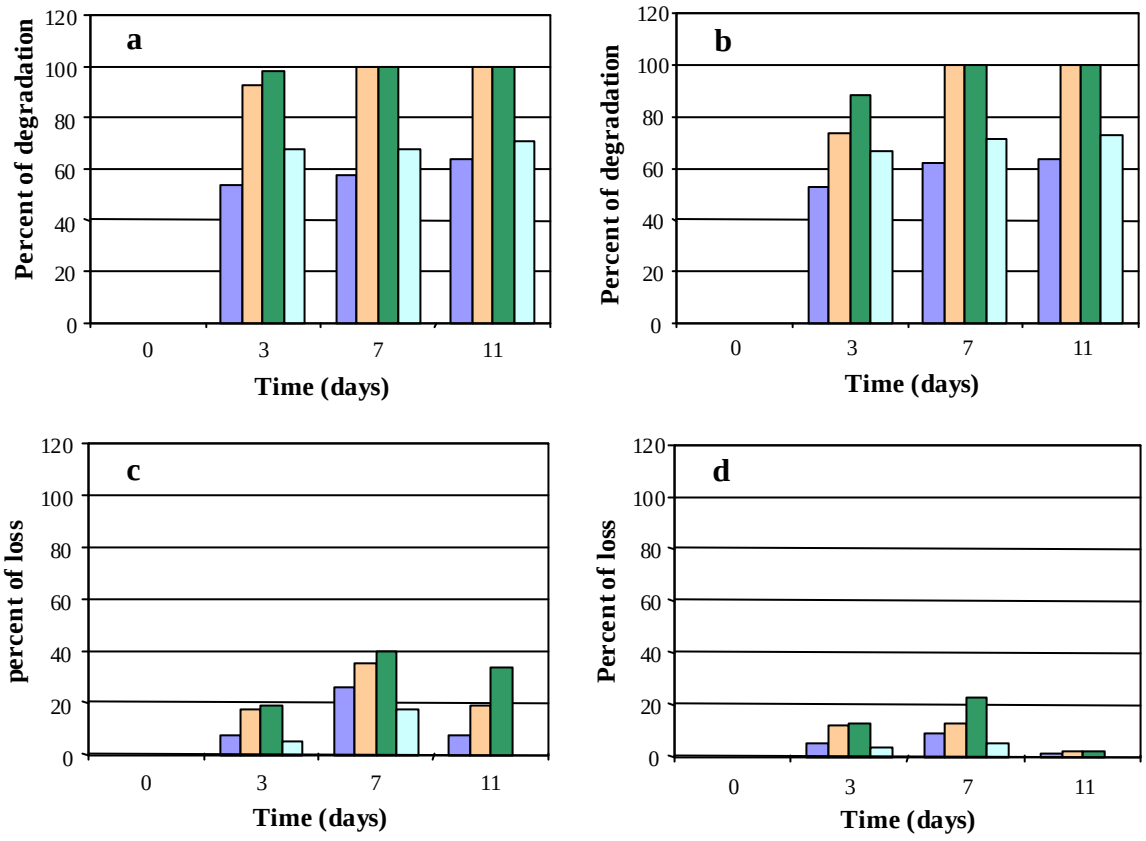


Figure 3.19. Percent of degradation of model compounds in a slurry experiment with Wadi Gaza microbial mats vs. time: a) 12 h light/12 h darkness; b) 24 h darkness; c) control with autoclaved biomass, 12 h light/12 h darkness; d) control without biomass, 24 h darkness. The data are presented in percent relative to the amount of compounds at zero time. ■ pristane; ■ dibenzothiophene; ■ phenanthrene; ■ *n*-octadecane.

the model compounds to some extent, can be excluded since the control treatment without mat material did not show the changes that were observed in the treatment with mat material.

The natural cyanobacterial mats inhabiting the chronically polluted site of Wadi Gaza appear to have a strong potential to effectively degrade petroleum model compounds. This supports previous studies, which indicated that chronic exposure to high levels of petroleum contamination results in microbial communities adapted to hydrocarbons with correspondingly higher concentrations of hydrocarbon-degrading bacteria (Carman et al., 1995, 1996). Biodegradation of the petroleum model compounds in this experiment is considered to be high, since after 7 days a residue of only 0-36% was left, depending on the type of compound. The aromatic compounds were completely degraded indicating complete accessibility of these compounds to the degrading bacteria. In contrast, the amount of aliphatic hydrocarbons decreased only slightly after 3 days and then remained constant up to 40 days. Apparently these compounds were only partially accessible to bacteria. It is likely that they were trapped by strong binding in the clay mineral interlayers, which inhibits desorption or direct biodegradation (Theng et al.,1998; Lahlou and Ortega-Calvo, 1999).

In our experiment 36 and 27% of pristane and *n*-octadecane were not degradable, respectively (Table 3.11 and Figures 3.19a and b). In a degradation experiment with organo-clay complexes in an aquarium with intact mats, it was observed that 37% of pristane and 40% of *n*-octadecane remained unaltered after four months (Grötzschel et al., 2002). A residual concentration of pristane and *n*-octadecane at this level was already observed in all earlier slurry degradation experiments with organo-clay complexes (Safi et al., 2001; Abed et al., 2002). This is in agreement with the concentration of the aliphatic hydrocarbons remaining at the end of the experiment described above.

In the present study the aromatic compounds were degraded faster and to a larger extent than the alkanes. This is in agreement with the observation that aromatic compounds in the seawater of the Persian Gulf were degraded at much higher rates than aliphatic compounds after the 1991 Gulf War oil spill (Fayad and Overton, 1995). In addition, the fact that the degradation rates of aromatic compounds were higher than those of aliphatic compounds is in accordance with laboratory experiments with intact microbial mats which extended over four months (Grötzschel et al., 2002).

3.14 Degradation of petroleum model compounds in a mesocosm experiment with intact cyanobacterial mats in the summer

3.14.1 Degradation of four model compounds

A contamination treatment experiment similar to that described in Chapter 3.12 was performed under field conditions in an outdoor experimental pond under the natural climate in Gaza, Palestine, with intact cyanobacterial mats from Wadi Gaza. The amount of each model compound on organo-clay added was 100 µg per cm^2 of mat surface (corresponding to 0.39, 0.37, 0.56 and 0.54 µmol of *n*-octadecane, pristane, phenanthrene and dibenzothiophene, respectively). Extraction of the suspension at day 0 yielded 77 to 90% of the theoretical amount of the four model compounds. This level of recovery was influenced by the experimental procedures such as sampling and chemical analysis. Three homogeneous looking sites on the mat surface were selected for sampling.

Degradation of pristane, dibenzothiophene, phenanthrene and *n*-octadecane was studied by their disappearance in the course of the experiments (Figure 3.20). In the control samples without cyanobacterial mats (Figure 3.21), the amounts and unaltered relative composition of the model compounds indicate that no degradation had occurred. A lower recovery and a slight decrease of phenanthrene and *n*-octadecane relative to the other model compounds was found in the control after 60 days; this pattern did not change until 100 days. Degradation started immediately after application of the four model compounds to the mats and slowed down in the course of the experiment (Figure 3.20 and Table 3.12). The aromatic substances dibenzothiophene and phenanthrene were degraded faster than the aliphatic compounds pristane and *n*-octadecane.

After seven days treatment, the averaged data of the samples taken from three different sites in the experimental pond show that 52% and 56% of dibenzothiophene and phenanthrene were removed, respectively (Figure 3.20). In the samples of days 30 and 40, 90% and 96% of these compounds were degraded, respectively. After 100 days the aromatic compounds phenanthrene and dibenzothiophene were completely removed. The aliphatic hydrocarbons pristane and *n*-octadecane were also degraded. In the case of pristane, 38% were degraded after seven days. Similarly, 38% of *n*-octadecane were removed. In the samples of day 30, ca. 54% of pristane and *n*-octadecane were degraded. After 70 days, the amounts of these two aliphatic compounds decreased to 21% and 18%, respectively. At the end of the experiment the remaining amounts of model compounds were below 0.5% of the

starting material for phenanthrene and dibenzothiophene and ca. 15% for pristane and *n*-octadecane (Figure 3.20).

Table 3.12. Average quantities (µg) of residual model compounds for the three sites in the mesocosm experiment performed in summer 2001 with cyanobacterial mats from Wadi Gaza and quantities in the aquarium control.

Time days	Average of the three sites				Aquarium control			
	Pr	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**
0	214.4	182.1	212.5	214.2	162.3	137.9	163.0	168.7
3	152.8	110.6	124.2	154.1	234.7	188.1	206.4	201.0
7	133.3	86.7	93.5	133.8	202.4	164.1	172.9	161.3
10	133.4	68.7	73.1	129.1	198.2	142.6	146.1	139.7
15	128.8	67.4	69.1	126.4	156.9	93.1	94.1	100.7
20	99.6	36.0	40.3	96.2	165.6	107.4	110.8	116.3
30	103.6	17.9	20.2	98.6	143.1	130.9	138.7	118.8
40	78.9	7.7	7.6	70.4	117.3	87.6	83.6	106.3
50	61.0	4.6	5.8	54.1	157.0	127.0	140.4	128.9
60	53.2	4.0	5.9	46.7	180.7	136.7	154.0	142.6
70	45.2	4.9	5.3	39.3	121.8	88.2	82.5	110.5
80	50.9	1.9	3.4	43.1	115.9	87.5	82.1	107.6
90	40.3	1.1	2.2	33.2	133.7	93.3	89.8	110.0
100	29.7	0.4	1.1	23.4	151.5	99.0	97.5	112.4

Pr: pristane; DBT: dibenzothiophene; Phen: phenanthrene and *n*-C_{18}: *n*-octadecane.

The degradation percentage of the four model compounds is different from one site to another. At site one 38 and 42% of dibenzothiophene and phenanthrene were degraded within seven days, respectively. After 40 days 94% of these compounds were removed. At the end of the experiment the dibenzothiophene was completely degraded and phenanthrene was degraded to 0.3%. In the case of the aliphatic compounds, 28% of pristane and *n*-octadecane were removed within the first week. In the samples of day 40, ca. 57% of pristane and *n*-octadecane were degraded. After 100 days the remaining amounts of pristane and *n*-octadecane were 10 and 7%, respectively (Figure 3.22a, Table 3.13).

After the first 40 days the extent of degradation of the four model compounds at site two was higher than at site one and three. After seven days, 55% and 59% of

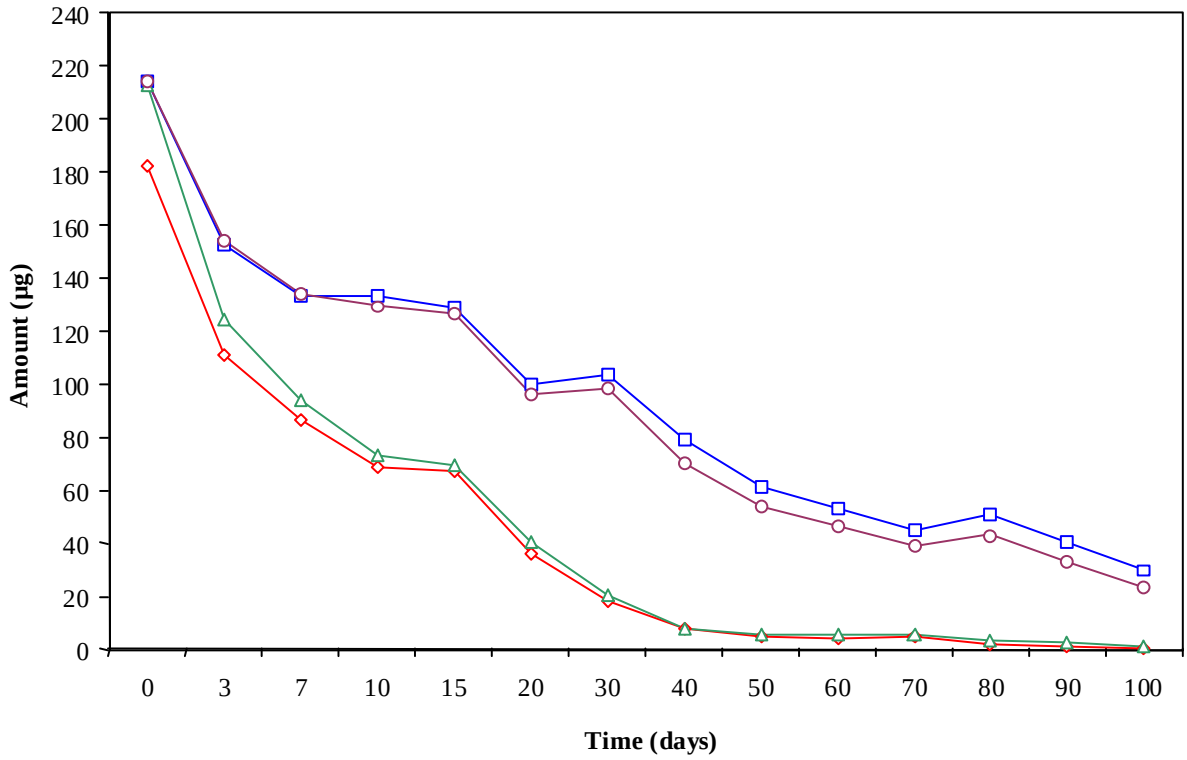


Figure 3.20. Biodegradation of petroleum model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed under field conditions in an outdoor experimental pond under the natural climate in Gaza. pristane; dibenzothiophene; phenanthrene; *n*-octadecane.

dibenzothiophene and phenanthrene were degraded, respectively. In the samples of day 40, 99% of these compounds were removed, this remained constant until 70 days. The remaining amounts of the aromatic compounds at the end of the experiment were below 0.5%. In the case of the aliphatic hydrocarbons, 76 and 80% of pristane and *n*-octadecane were degraded within 40 days, respectively. The degradation of these compounds increased to 88% and 90% at day 70 and remained constant until the end of the experiment (Figure 3.22b and Table 3.13).

At site three, 68% and 70% of dibenzothiophene and phenanthrene were degraded within the first week, respectively. In the samples of day 40, 94% of these compounds were removed. Less than 1% of the starting amounts of phenanthrene and dibenzothiophene remained at the end of the experiment. In the first week, 54% of the aliphatic compounds, pristane and *n*-octadecane were degraded. After 40 days, 58% and 65% of these compounds were removed, respectively. In the samples of 70 days, 67% and 71% of pristane and *n*-octadecane were degraded, respectively. At the end of the experiment, 18% and 22% of the starting amounts of *n*-octadecane and pristane remained, respectively (Figure 3.22c and Table 3.13).

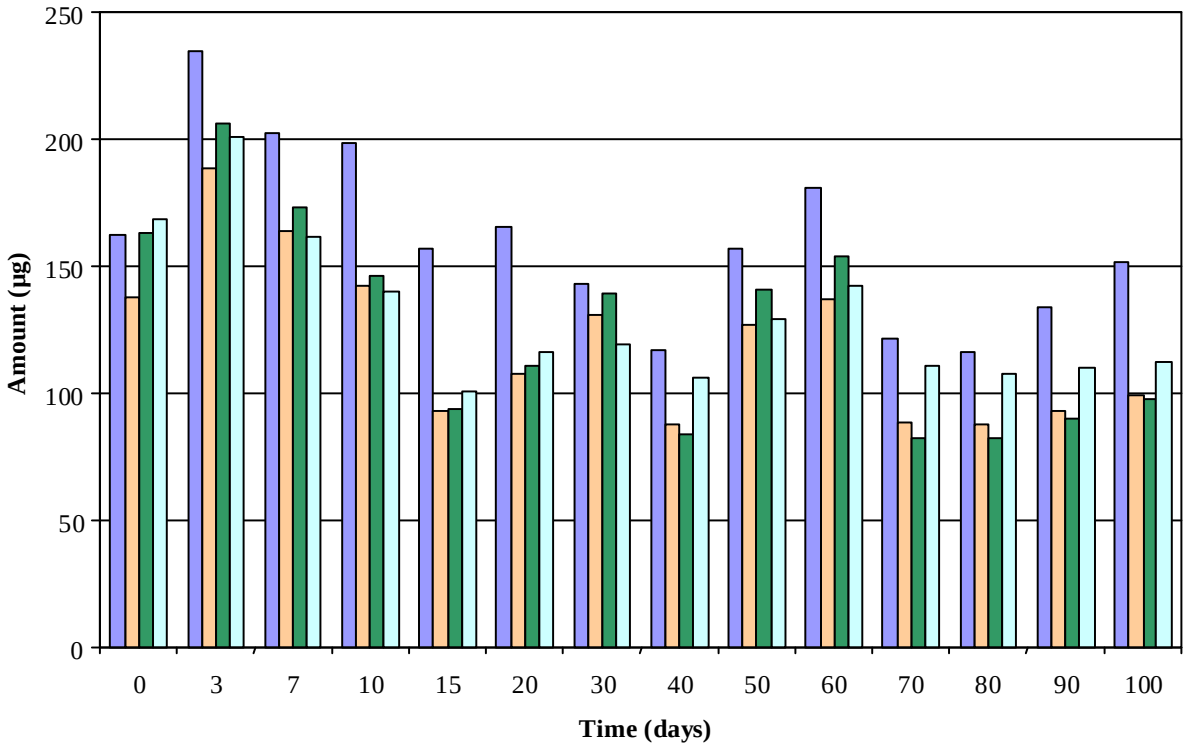


Figure 3.21. Quantities (µg) of petroleum model compounds in the aquarium control without microbial mats as part of the degradation experiment performed under field conditions in outdoor ponds under the natural climate in Gaza. ▫ pristane; ▫ dibenzothiophene; ▫ phenanthrene; ▫ *n*-octadecane.

In the first week, the degradation of the four model compounds at site three was faster than at sites one and two. The aromatic compounds dibenzothiophene and phenanthrene were degraded faster than the aliphatic compounds at all sites in the course of the experiment. More than 94% of phenanthrene and dibenzothiophene were removed at the three sites within 40 days. At the end of the experiment the aromatic compounds had nearly disappeared at all sites. The complete bioavailability of the aromatic compounds may be due to their structures and shapes. El-Nahhal and Safi (2004) have reported that adsorption to the organo-clay complex occurs via interaction between the aromatic rings of the phenanthrene molecule and those of the organo-clay complex. In slurry degradation experiments with organo-clay complexes it was observed that aromatic compounds were degraded completely and faster than the aliphatic compounds (Safi et al., 2001; Abed et al., 2002). In addition, it has been observed that aromatic compounds in degradation experiments with organo-clay complexes in an aquarium with intact microbial mats from Eilat were degraded faster and to a larger extent than the alkanes (Grötzschel et al., 2002). This is in agreement with the faster degradation rate of aromatic compounds observed in the present study.

Table 3.13. Quantities (µg) of residual model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed in summer 2001. Pr: pristane; DBT: dibenzothiophene; Phen: phenanthrene and *n*-C_{18}: *n*-octadecane.

Time days	Site 1				Site 2				Site 3			
	Pr	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**
0	238.0	185.3	212.0	235.8	225.0	212.4	253.8	226.2	180.3	148.7	171.8	180.7
3	163.8	136.5	162.8	164.8	166.8	101.1	115.6	171.8	127.8	94.2	94.2	125.6
7	172.0	115.6	123.8	170.6	145.2	96.4	104.9	145.2	82.8	48.2	51.8	85.7
10	172.2	107.1	112.4	176.6	172.5	64.2	69.7	157.2	55.5	34.8	37.1	53.6
15	175.4	88.4	89.3	177.0	135.1	74.1	73.6	126.4	75.9	39.6	44.5	75.8
20	169.4	58.9	62.6	163.5	58.8	24.4	28.0	58.1	70.6	24.6	30.2	67.0
30	106.4	19.8	21.4	102.2	79.9	18.0	19.6	71.1	124.6	15.7	19.8	122.4
40	108.2	11.2	12.5	102.3	53.3	3.0	2.0	44.6	75.0	8.8	8.2	64.2
50	57.7	4.5	8.7	52.6	53.8	3.8	2.9	48.0	71.7	6.0	5.8	61.7
60	54.6	4.5	7.2	46.2	45.2	1.6	3.0	39.8	59.8	5.9	7.5	54.0
70	48.4	6.8	6.0	41.7	28.0	2.1	2.6	23.4	59.1	5.7	7.2	53.0
80	55.8	1.3	1.3	48.3	31.8	2.0	4.1	24.0	65.1	2.4	4.8	57.0
90	39.8	0.7	1.0	32.9	28.3	1.2	2.6	21.7	52.7	1.5	3.1	45.2
100	23.7	0.0	0.6	17.5	24.9	0.5	1.2	19.4	40.4	0.6	1.5	33.4

In the case of the aliphatic compounds, the remaining amounts of pristane and *n*-octadecane at site one and two were smaller than at site three. A non-degradable residual concentration of aliphatic hydrocarbons was already observed in all earlier degradation experiments with organo-clay complexes (Safi et al., 2001; Abed et al., 2002; Grötzschel et al., 2002). In the present study, the association of the petroleum model compounds with the hydrophobic clay may have influenced the bioavailability of the different substances. Guerin and Boyd (1997) demonstrated that bacteria have different abilities to degrade hydrophobic xenobiotic compounds associated with solid material. Differences in the accessibility of sorbed compounds may depend on whether microorganisms are able to degrade these compounds directly from the surface of the carrier material or whether they are dependent on the spontaneous desorption of the substances (Calvillo and Alexander, 1996: Tang et al., 1998).

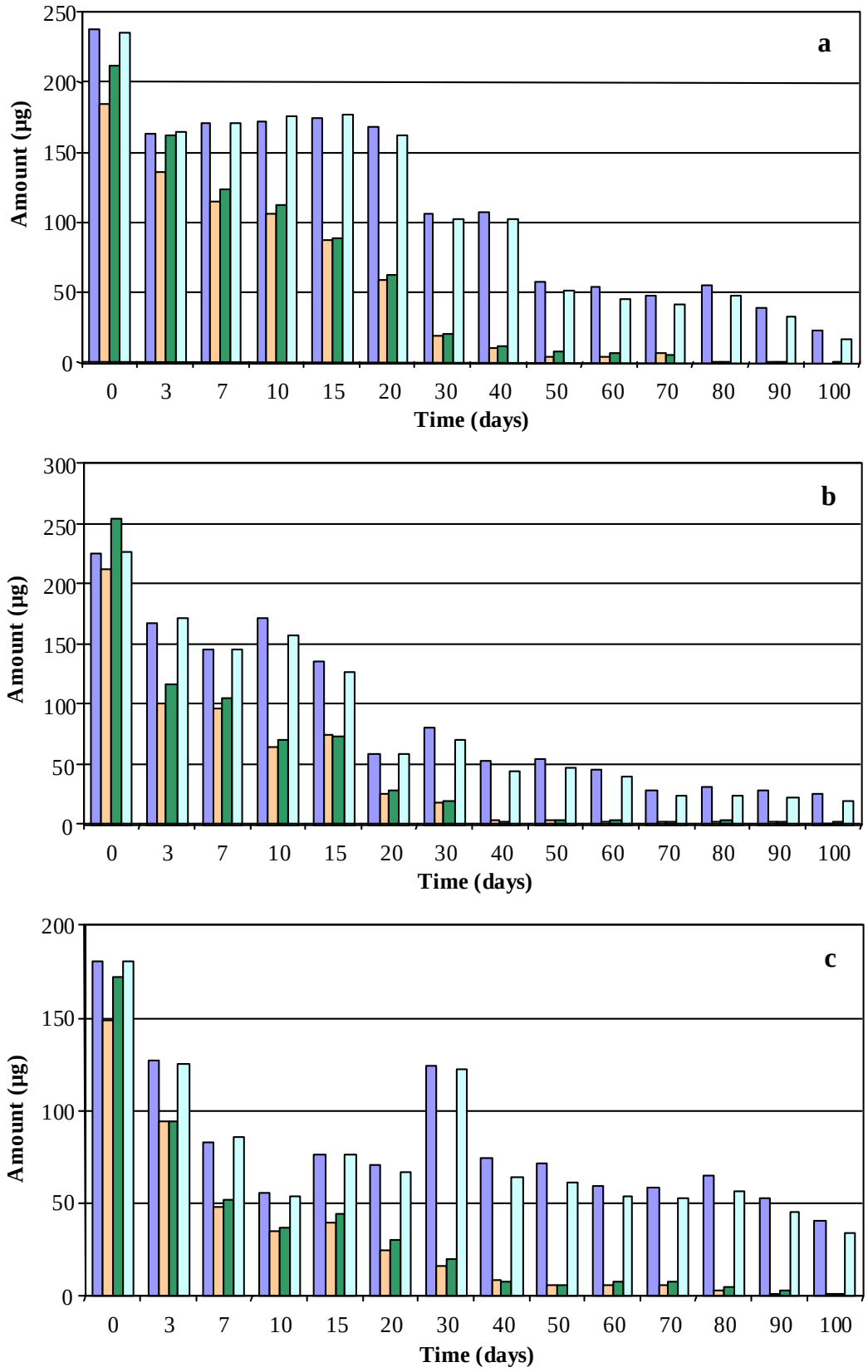


Figure 3.22. Summer degradation of petroleum model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed under field conditions in outdoor experimental ponds under the natural climate in Gaza. a: site one; b: site two; c: site three. ▫ pristane; ▫ dibenzothiophene; ▫ phenanthrene; ▫ *n*-octadecane.

According to GC-MS analysis no peaks of newly formed substances appear in the chromatograms. This means that no significant accumulation of possible metabolites or degradation products could be observed in this experiment. This suggests that degradation of the petroleum model compounds has led to complete mineralization.

Complete mineralization may be favored by the presence of a complex microbial community. Radwan et al. (1995) and Korda et al. (1997) reported that effective bioremediation of petroleum contamination requires a mixture of populations consisting of different genera each capable of metabolizing specific petroleum compounds.

The differences in the degradation activity at the three sites are due to the inhomogeneity of the mats. There are areal differences in the composition of the microbial community in the mat. The biovolume of unicellular cyanobacteria in the control section was significantly higher than that in the section with model compound. In contrast, the biovolume of Oscillatoria and thin filamentous cyanobacteria in the treated section were significantly higher than in the control section (M. Yassin, unpublished data). Abed et al. (2002) showed that shifts in the cyanobacterial community composition and the development of new populations occurred in the slurry degradation experiments. The fact that ≥94% of dibenzothiophene and phenanthrene were degraded at the three sites within 40 days may be due to an enrichment of the mats with species specialized in the degradation of these compounds.

The degradation rate of the four model compounds decreased with decreasing residual amount of the compounds. This may indicate that the petroleum model compounds had a toxic effect on the growth of certain microorganisms. Many studies have demonstrated that crude oil contains constituents which are inhibitory to cyanobacteria even at low concentrations (Radwan and Al-Hasan, 2000 and references therein).

In an oil degradation experiment Al-Gounaim et al. (1995) demonstrated that the biodegradation activity of the microbial population of polluted samples decreased with decreasing amount of residual oil. This is in agreement with the results of the present study. The decreasing concentrations of the petroleum model compounds (Table 3.12) , as well as the changes in their relative amounts, indicate a high biodegradation efficiency, although with compound-specific differences, in the course of the experiment. Major photooxidation can be excluded as a mechanism of compound disappearance since the control treatment without mat material did not show changes similar to those found in the treatment with mat material.

3.14.2 Measurement of water depth, temperature, salinity and pH during the summer experiment

Water depth, temperature, salinity and pH values in the experimental section pond were monitored three times a week and during the sampling time for the purpose of compensating evaporation by freshwater maintaining a water column of about 30 cm height and avoiding extreme salinity excursions. The temperature range was between 29 and 35°C. The water depth decreased and the salinity increased with increasing temperature due to evaporation. During the experiment the salinity range was between 4.1 and 7.1%. The pH values were between 8.1 and 9.5 (Figure 3.23a and Table 3.14). In the case of the aquarium control, the temperature range was between 25 and 42°C, and the salinity varied between 4.3 and 10%. At day 20, 50% of the water had evaporated from the aquarium due to the high temperature of the glass container. The pH values in the aquarium varied between 7.4 and 9.9 (Figure 3.23b).

Table 3.14. Minimum and maximum values of water depth, temperature, salinity and pH value during degradation of model compounds in mesocosm experiments with cyanobacterial mats from Wadi Gaza performed in summer and winter in Gaza.

Experiment	Water depth (cm)	Temperature (°C)	Salinity (%)	pH value
Summer	17-30	29-35	4.1-7.1	8.1-9.5
Winter	30-57	11-30	2.4-5.1	5.7-7.5

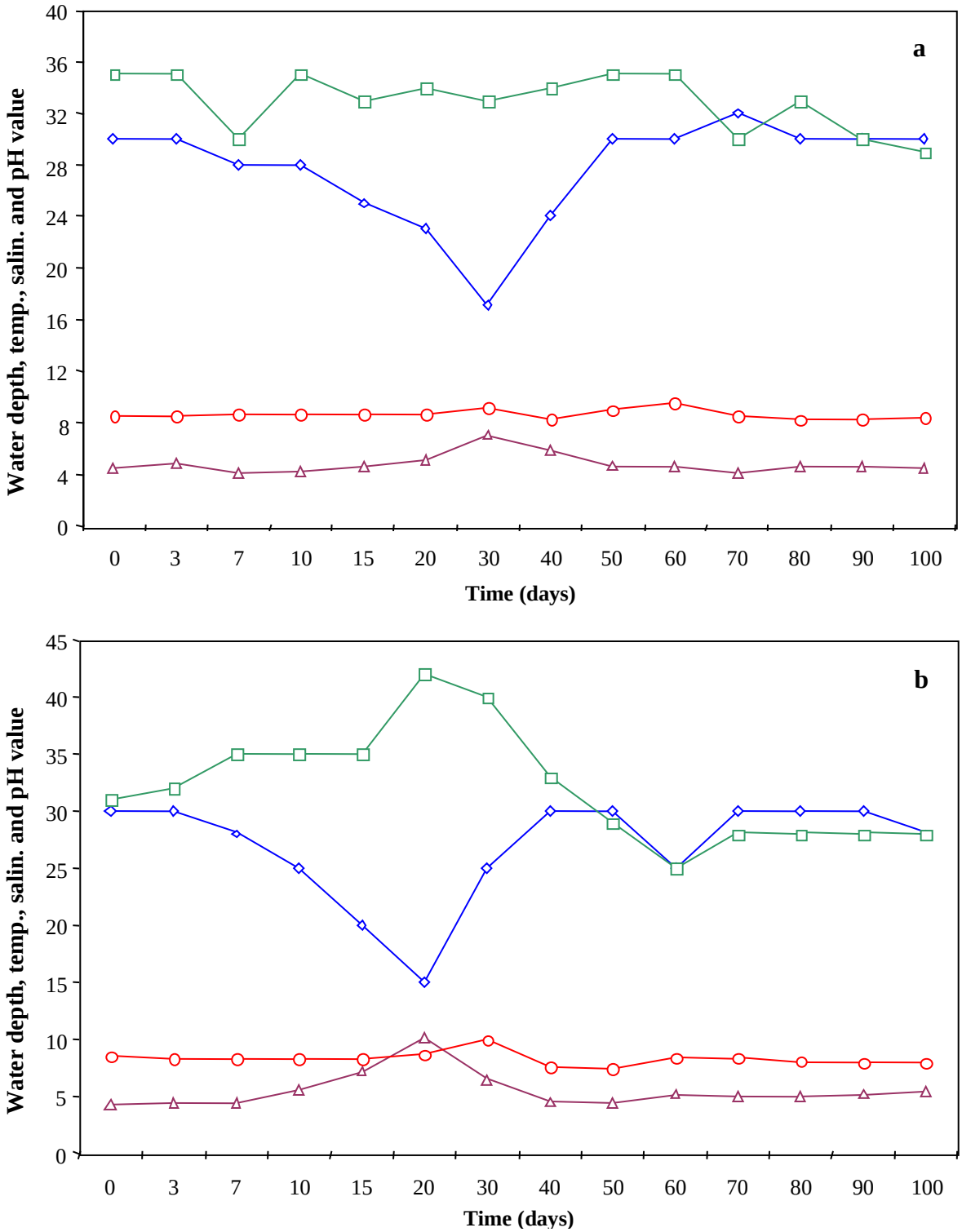


Figure 3.23. Water depth, temperature, salinity and pH values during experimental degradation of petroleum model compounds in a mesocosm with cyanobacterial mats from Wadi Gaza performed in summer. a: experimental section pond and b: aquarium control without microbial mats. Both a and b under field conditions in outdoor experiment under the natural climate in Gaza. ◇ water depth (cm); □ temperature (°C); △ salinity (%); ○ pH values.

3.15 Degradation of petroleum model compounds in a mesocosm experiment with intact cyanobacterial mats in the winter

3.15.1 Degradation of four model compounds

A second experiment was performed from 30th October 2001 till 8th February 2002 in order to investigate the effect of repeated exposure to contaminants and the performance of the mats during winter time. The same procedure as described in chapter 3.14 for the summer experiment was repeated in the winter. The same amount of organo-clay complex loaded with model compounds was added to the same pond after the 100 days period of the summer experiment.

Degradation of the four petroleum model compounds applied to the mats started immediately and slowed down in the course of the experiment (Figure 3.24 and Table 3.15). The aromatic compounds phenanthrene and dibenzothiophene and the aliphatic substances *n*-octadecane and pristane were degraded in the course of the experiment nearly to the same level and at the same rate as in the summer.

Table 3.15. Average quantities (µg) of residual model compounds for the three sites in the mesocosm experiment with cyanobacterial mats from Wadi Gaza conducted in winter 2001/2002.

Time (days)	**0**	**3**	**7**	**10**	**15**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Pr	259.5	209.8	144.0	144.5	144.9	130.3	106.1	100.3	95.7	96.7	88.7	82.9	61.5	42.9
DBT	116.8	82.0	71.1	75.2	75.2	71.3	54.8	47.3	45.3	48.4	36.8	31.4	31.7	18.8
Phen	135.4	91.1	83.0	87.9	88.7	74.8	58.9	54.5	47.3	49.6	37.0	31.7	33.3	20.7
***n*-C_{18}**	234.5	187.0	131.3	119.5	120.1	114.2	95.5	87.5	79.3	87.5	74.4	67.6	50.9	34.8

Pr: pristane; DBT: dibenzothiophene; Phen: phenanthrene and *n*-C_{18}: *n*-octadecane.

The averaged data of the three sites show that 44% of dibenzothiophene and phenanthrene were degraded within seven days (Figure 3.24). In the samples of day 40, 60% of these compounds were removed. After 100 days, 85% the aromatic compounds phenanthrene and dibenzothiophene were degraded. In the case of the aliphatic compounds, 44% of pristane and *n*-octadecane were removed within seven days. The degradation of these compounds increased to 62% at day 40. At the end of the experiment, the residual amounts of the aliphatic compounds *n*-octadecane and pristane was 15% of the starting amounts (Figure 3.24).

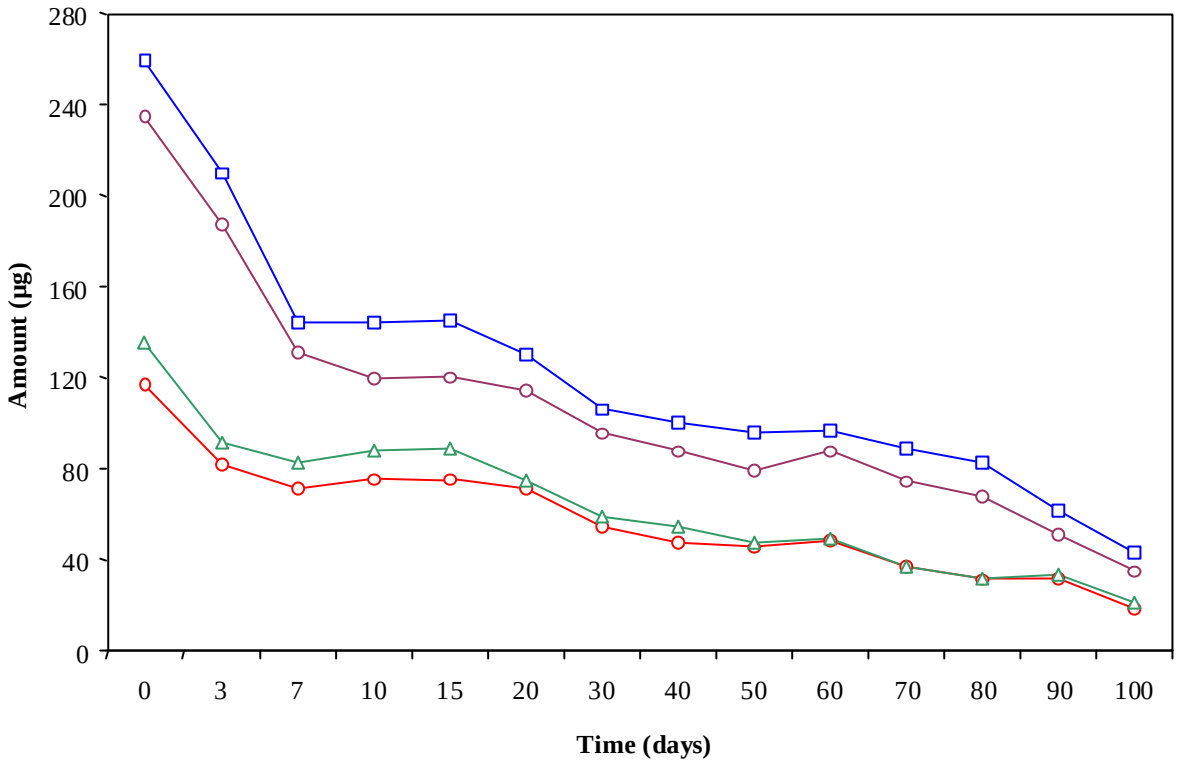


Figure 3.24. Biodegradation of petroleum model compounds in a winter mesocosm experiment with cyanobacterial mats from Wadi Gaza performed under field conditions in an outdoor experimental pond under the natural climate of Gaza. The amounts represent the average of three sampling sites. —□— pristane; —◇— dibenzothiophene; —△— phenanthrene; —○— *n*-octadecane.

As in the summer experiment, the degradation percentage of the four model compounds is different from one site to another. Table 3.16 presents the quantities (µg) of the residual petroleum model compounds at the three sites during the winter experiment. At site one, 51 and 55% of dibenzothiophene and phenanthrene were degraded within seven days, respectively. After 40 days 64% of these compounds were removed. In the samples of day 70**,** 69% and 77% of dibenzothiophene and phenanthrene were degraded, respectively. At the end of the experiment 85% of phenanthrene and dibenzothiophene were removed. In the case of the aliphatic hydrocarbons, 58% of pristane and *n*-octadecane were degraded within the first week, and the residual amount remained nearly constant until day 70. After 100 days, 86% of pristane and *n*-octadecane were degraded (Figure 3.25a). At this site, equal amounts of aliphatic and aromatic compounds were removed at the end of the experiment.

At site two, 31% of phenanthrene and 38% of dibenzothiophene were removed within seven days. In the samples of days 40 and 70, their is no significant difference in the degradation of these compounds between sites two and one. At the end of the experiment, the remaining amounts of the aromatic compounds were 12% of the starting amounts (Figure 3.25b).

Table 3.16. Quantities (µg) of residual model compounds in the mesocosm experiment with cyanobacterial mats from Wadi Gaza conducted in winter 2001/2002.

Time days	Site 1				Site 2				Site 3			
	Pr	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**
0	270.1	124.3	154.4	217.9	182.3	128.1	141.9	172.6	326.1	98.1	109.9	313.0
3	245.2	60.0	66.8	222.5	146.3	104.7	112.7	136.9	237.9	81.2	93.7	201.5
7	105.7	61.2	69.7	90.6	178.1	79.9	97.8	171.0	148.0	72.2	81.5	132.4
10	129.9	56.3	70.8	96.3	160.9	82.7	98.3	141.2	142.6	86.8	94.6	121.0
15	101.8	59.3	67.3	82.3	188.1	91.2	102.8	170.5	144.8	75.2	95.9	107.6
20	114.7	61.0	65.1	97.3	153.1	89.3	90.9	138.8	123.0	63.5	68.3	106.6
30	112.3	51.6	53.2	96.4	104.0	56.6	62.1	94.9	101.9	56.1	61.5	95.2
40	114.1	44.3	52.8	97.0	100.6	54.4	61.9	93.4	86.3	43.3	48.9	72.1
50	97.4	49.2	54.2	86.2	107.2	52.2	54.3	88.1	82.7	34.5	33.5	63.7
60	123.4	56.0	53.8	113.4	91.1	55.2	61.9	84.8	75.7	34.0	33.0	64.2
70	112.2	37.9	35.6	95.9	83.3	39.5	39.8	71.3	70.4	33.0	35.5	56.1
80	95.4	33.0	33.5	83.7	82.1	32.7	36.0	75.9	71.2	28.6	25.7	43.1
90	53.2	34.3	34.7	47.2	60.8	33.0	36.2	56.8	70.4	27.8	28.9	48.8
100	35.2	18.9	20.4	31.6	40.8	13.8	17.3	33.0	52.8	23.7	24.5	39.9

Pr: pristane; DBT: dibenzothiophene; Phen: phenanthrene and *n*-C_{18}: *n*-octadecane.

No degradation was observed for the aliphatic compounds *n*-octadecane and pristane in the first week at site two. After 40 days, 45% of pristane and *n*-octadecane were degraded. The extent of degradation of these compounds increased to 54% and 59% at day 70, respectively. At the end of the experiment, 78% and 81% of pristane and *n*-octadecane were removed, respectively (Figure 3.25b).

At site three, 26% of dibenzothiophene and phenanthrene were degraded within the first week. After 40 days 56% of these compounds were removed. In the samples of day 70, 66% of the aromatic compounds were degraded. After 100 days, the remaining amounts were below 25% of the starting amounts of phenanthrene and dibenzothiophene. In the case of the aliphatic compounds, the removed amounts of pristane and *n*-octadecane were nearly similar to those at site one within the first week. In the samples of day 40, 74% of these compounds were degraded. After 70 days, 78% and 82% of pristane and *n*-octadecane were removed, respectively. At the end of the experiment, 16% of the starting amounts of *n*-octadecane and pristane remained (Figure 3.25c).

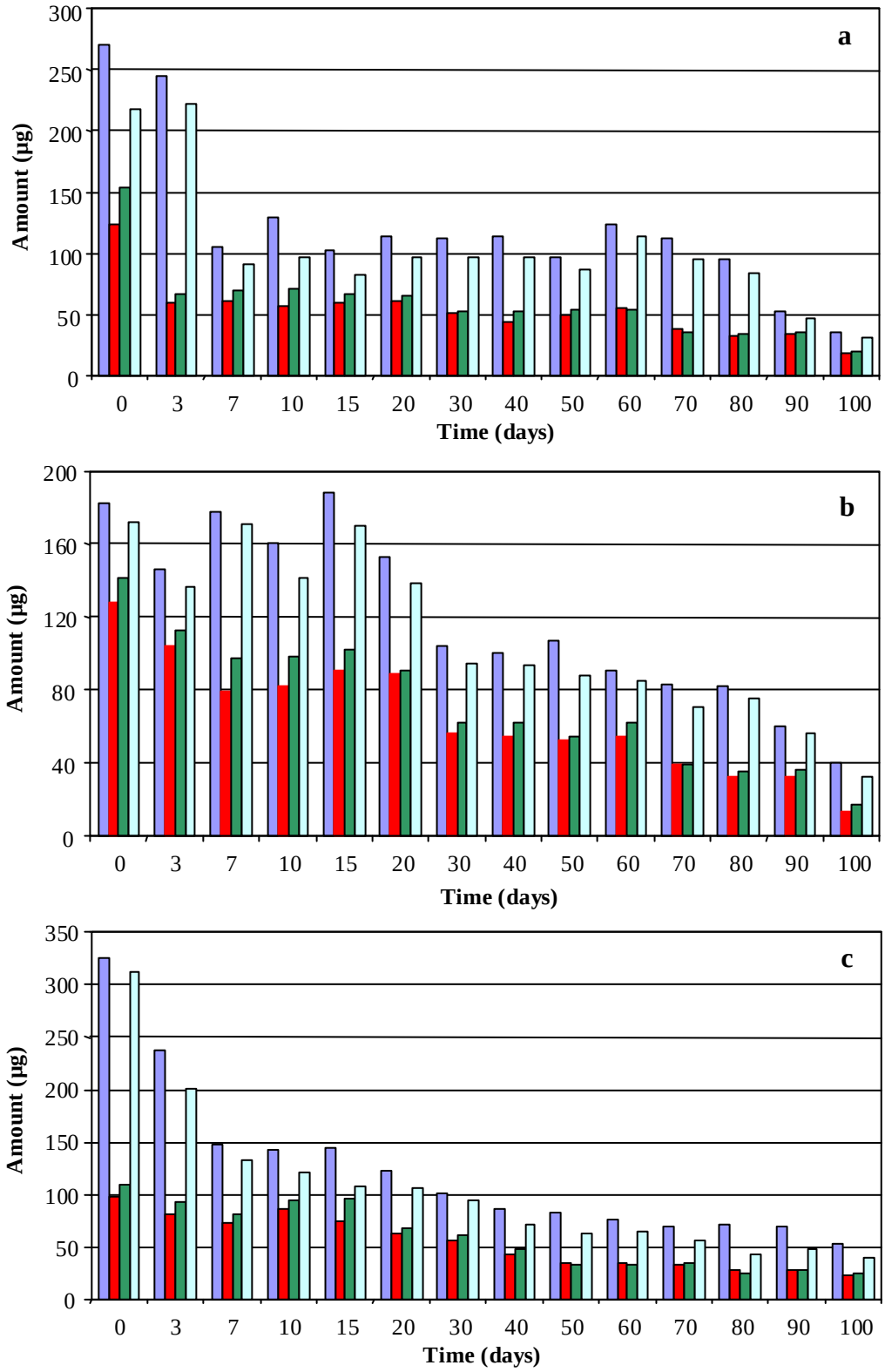


Figure 3.25. Biodegradation of petroleum model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed under field conditions in an outdoor experimental pond under the natural climate in Gaza. a: site one; b: site two; c: site three. ▫ pristane; ▫ dibenzothiophene; ▫ phenanthrene; ▫ *n*-octadecane.

In the first week, the degradation of the aromatic compounds at site one was significantly higher than that at sites two and three. After 100 days the removed amounts of these compounds at site one and site two were higher than at site three. The degradation percentage of the aliphatic compounds was nearly the same at sites one and three. Surprisingly, there was no significant degradation of these compounds at site two in the first three weeks. At site three, the removed amounts of the aliphatic compounds were significantly higher than at sites one and two in the first 40 days. The degradation of the four model compounds at site one was faster than at sites two and three in the first week. At the end of the experiment the amounts of the aromatic compounds were below 15% of the starting amounts at both sites one and two. At site three the remaining amounts of these compounds were below 25%. In the case of the aliphatic compounds, the remaining amounts of pristane and *n*-octadecane were below 16% at sites one and three and below 23% of the starting amounts at site two.

The differences in the degradation of the four model compounds among the three sites were also observed in the winter experiment. These results suggest that the abundance of some specific species in the cyanobacterial mats was different at the three sites. However, a characterization of the bacterial community and of community changes has not been performed yet. The highest degradation percentages of 55% and 58% were observed for the aliphatic and aromatic compounds at site one within the first week, respectively. This suggest that site one is rich in bacteria that are able to degrade the model compounds. At all sites, ≥85% of dibenzothiophene and phenanthrene were removed in the course of the experiment. This may indicate that the microbial mats are enriched with species specialized in degrading these compounds.

3.15.2 Measurement of water depth, temperature, salinity and pH during the winter experiment

The same measurements as described above for the summer experiment were repeated in winter. The temperature range was between 11 and 30°C. The water height increased and the salinity decreased with decreasing temperature due to rainfall and changes of the weather. The salinity range was between 2.4 and 5.1% in the course of the experiment. At the end of the experiment, the water depth was 57 cm and the salinity was 2.4%. The pH values ranged between 5.7 and 7.5 (Figure 3.26a and Table 3.14). In the case of the aquarium control, the

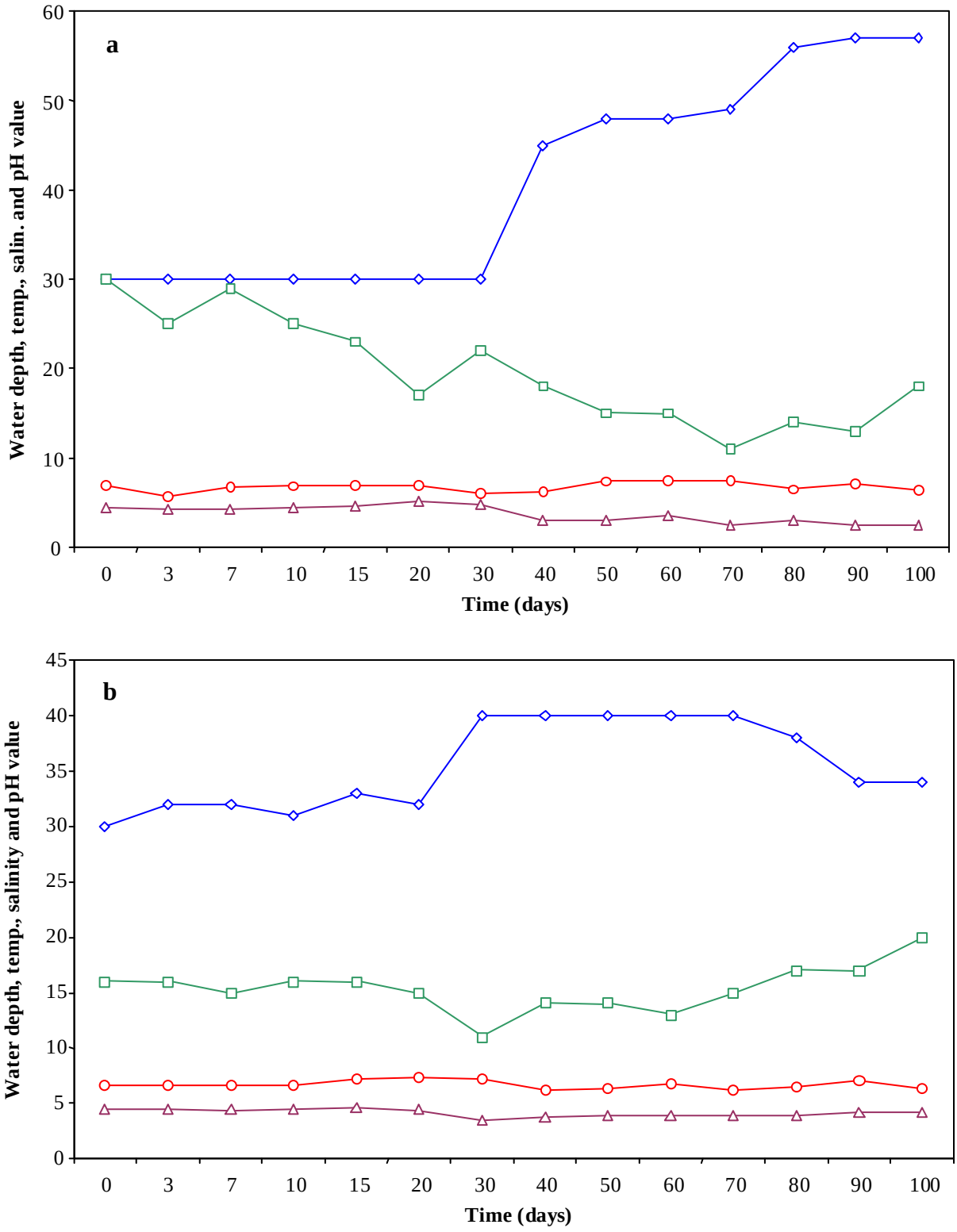


Figure 3.26. Water depth, temperature, salinity and pH values for degradation of petroleum model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed in winter 2001/2002. a: experimental section pond and b: aquarium control without microbial mats. Both a and b under field conditions in an outdoor experiment under the natural climate in Gaza. ◇ water depth (cm); □ temperature (°C); △ salinity (%); ○ pH values.

temperature range was between 11 and 20°C, the salinity between 3.4 and 4.6% and the pH values ranged between 6.1 and 7.3 (Figure 3.26b).

3.15.3 Comparison between summer and winter experiments

There is a significant difference in the degradation of the aromatic compounds between summer and winter in the course of the experiment. In the first week, 53% and 39% of the these compounds were degraded in summer and winter, respectively. In the samples of day 40, 96% and 60% of these compounds were degraded in summer and winter, respectively. After 40 days, the degradation of the aromatic compounds slowed down in both summer and winter experiments. This may indicate that the contaminants had reduced the activity of certain microorganisms responsible for the degradation of the aromatic compounds. Pollution with these compounds inhibits photosynthesis and growth, reduces enzyme activity and microbial biomass and induces changes in the cyanobacterial species composition (Megharaj et al., 2000). The aromatic compounds were completely degraded within 70 days in the summer experiment. In the winter experiment, 70% of these compounds were degraded within 70 days and 15% of the starting amounts remained at the end of the experiment. In the case of the aliphatic compounds, the degradation percentages of pristane and *n*-octadecane were higher in summer than in winter at day 70. At the end of the experiment, ca 15% of the starting amounts of these compounds remained in both summer and winter experiments. The re-exposure of the mats to the contaminants did not speed up degradation of the four model compounds. These results suggest that light and temperature (29-35°C) enhance the degradation activity of the cyanobacterial mats especially for the aromatic compounds as observed in the summer experiment.

3.15.4 Growth of the cyanobacterial mats

At the beginning of the summer and winter experiments, the appearance of the mat surfaces was dark green in both the contaminated pond section with petroleum model compounds and the control mats without the contaminants. A significant difference in the growth of the mats between the treatment and the control were observed during the experiment (Figure 3.27a and b). At the end of the experiment the mats showed a brown green color in the treatment, and the mat color had remained the same dark green in the control. The Oscillatoria and thin filamentous cyanobacteria were more abundant in the treated section than in the control section. In contrast, the unicellular cyanobacteria in the control section were more abundant than in the treated section (M. Yassin, unpublished data). Abed et al. (2002) showed that Wadi Gaza cyanobacterial mats contain a significant hidden diversity of microorganisms which may contribute to the degradation of different petroleum compounds.

The development of the microbial mats in the presence of the model compounds indicates that the mats degraded these compounds and used it as a sole carbon source. Many studies, e.g. by Hanson et al. (1997) and Cha et al. (1999), showed that microorganisms can use hydrocarbons as their sole carbon source. Degradation of petroleum model compounds was accompanied by changes in both cyanobacterial and bacterial communities in a slurry experiment. The development of certain populations was attributed directly to the incubation with the model compounds and thus believed to play a role in the metabolism of those compounds (Abed et al., 2002).

3.15.5 Degradation kinetics

It is found from regression analysis that the data of degradation of four model compounds fit first-order kinetics as described by Segel (1976). The equation is $\ln C_t = \ln C_0 - Kt$, where C_0 represents the initial concentration, C_t represents the concentration at time t, t represents the time in days and K represents the decay rate constant. The larger the value of K, the faster is the degradation process.

First-order rate constants (K), correlation coefficient (r^2) and the time required for 50% degradation of the tested compounds (Dt_{50}) calculated for the degraded four model compounds are presented in Table 3.17. It can be seen that the degradation patterns follow an exponential degradation in all cases (Figures 3.28). Linear regression based on the logarithm of the concentration (e.g. [K-pristane]) was performed to detect any differences between experiments and compound groups. Figures 3.28 and 3.29 show that all differences are in the

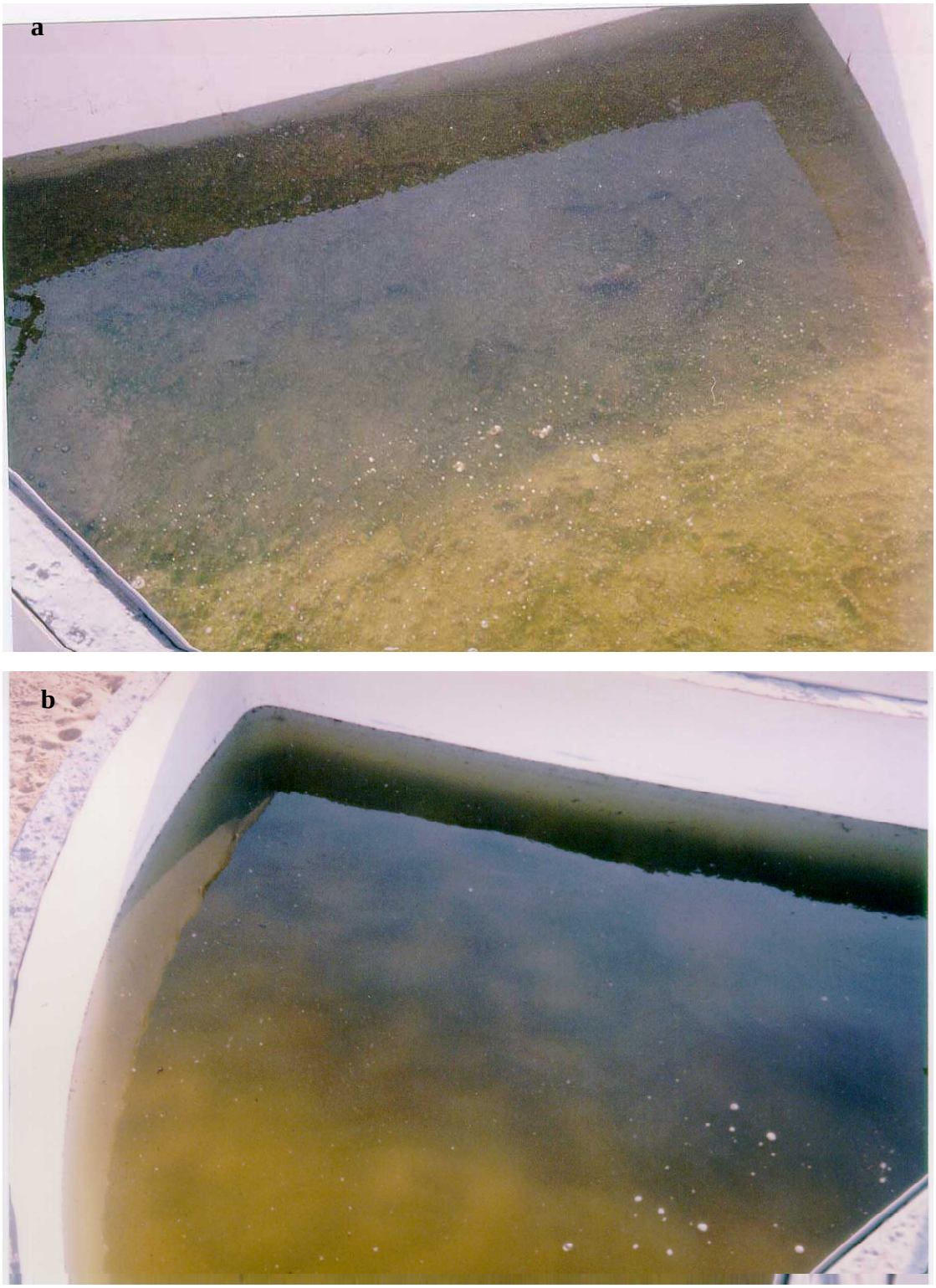


Figure 3.27. Growth of cyanobacterial mats following degradation of petroleum model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed under field conditions in outdoor experimental ponds in Gaza. a: cyanobacterial mats with organo-clay complex. b: control section, cyanobacterial mats without organo-clay complex.

range of the statistical error , this means degradation in all experiments truly follows first-order kinetics.

The correlation coefficient values (r^2) are higher than 0.9 and 0.87 for all compounds in summer and winter experiments, respectively (Table 3.17 and Figures 3.28 and 3.29). The degradation rate constant (K) for phenanthrene calculated from the above equation is higher than that for *n*-octadecane or pristane. These data suggest that degradation of phenanthrene (aromatic compound) occurs more quickly than that of aliphatic compounds (*n*-octadecane, pristane).

Table 3.17. First-order degradation rate constant, correlation coefficient and degradation half-life time of petroleum model compounds in a mesocosm experiments with cyanobacterial mats.

Experiment		**Pristane**	**Dibenzothiophene**	**Phenanthrene**	***n*-Octadecane**
Summer	K	0.0173	0.047	0.0496	0.0188
	r^2	0.96	0.95	0.92	0.91
	Dt_{50}	26	10	10	24
Winter	K	0.0127	0.0136	0.0147	0.0135
	r^2	0.87	0.92	0.94	0.88
	Dt_{50}	27	31	29	25

K = degradation rate constant, r^2 = correlation coefficient and Dt_{50} = degradation half-life time.

The decay rate constants (K) of the four model compounds calculated from the regression equation show that the values for the aromatic compounds are higher than those for the aliphatic compounds (Table 3.17). These data show that the degradation rate of aliphatic compounds is slower than that of aromatic compounds. The relatively high degradation rates for the aromatic compounds may be due to their greater accessibility to the bacteria. These results suggest that degradation is a function of desorption of the model compounds. In biodegradation of phenanthrene in soil experiment, at a concentration of 5 µg/g, the degradation rate constant was measured at 0.0269 h^{-1} with a half-life time of 25.8 h (Chang et al., 2001). Whereas in river sediment biodegradation rate constants and half-lives time ranged from 0.12 to 1.13 day^{-1} and 0.61 to 5.78 days, respectively (Yuan et al., 2001). The calculated half-life times of phenanthrene and dibenzothiophene in slurry experiment with Gaza mats were less than 3.5 days for both (Abed et al., 2002), whereas in an experiment with intact mats from Eilat, they were 74.3 and 72.4 days respectively (Grötzschel et al., 2002).

Comparing the Dt_{50} value (time required for degradation of 50% of the parent compound) shows a short degradation time for phenanthrene and dibenzothiophene (10 days) in the summer experiment. In the winter experiment, the aliphatic compounds pristane and *n*-octadecane had shorter degradation times of, 27 and 25 days, respectively (Table 3.17).

Statistical analysis showed significant differences in Dt_{50} for the aromatic compounds between summer and winter. This may indicate that light and temperature enhance some species in the microbial mats specialized in degrading these compounds in the summer experiment.

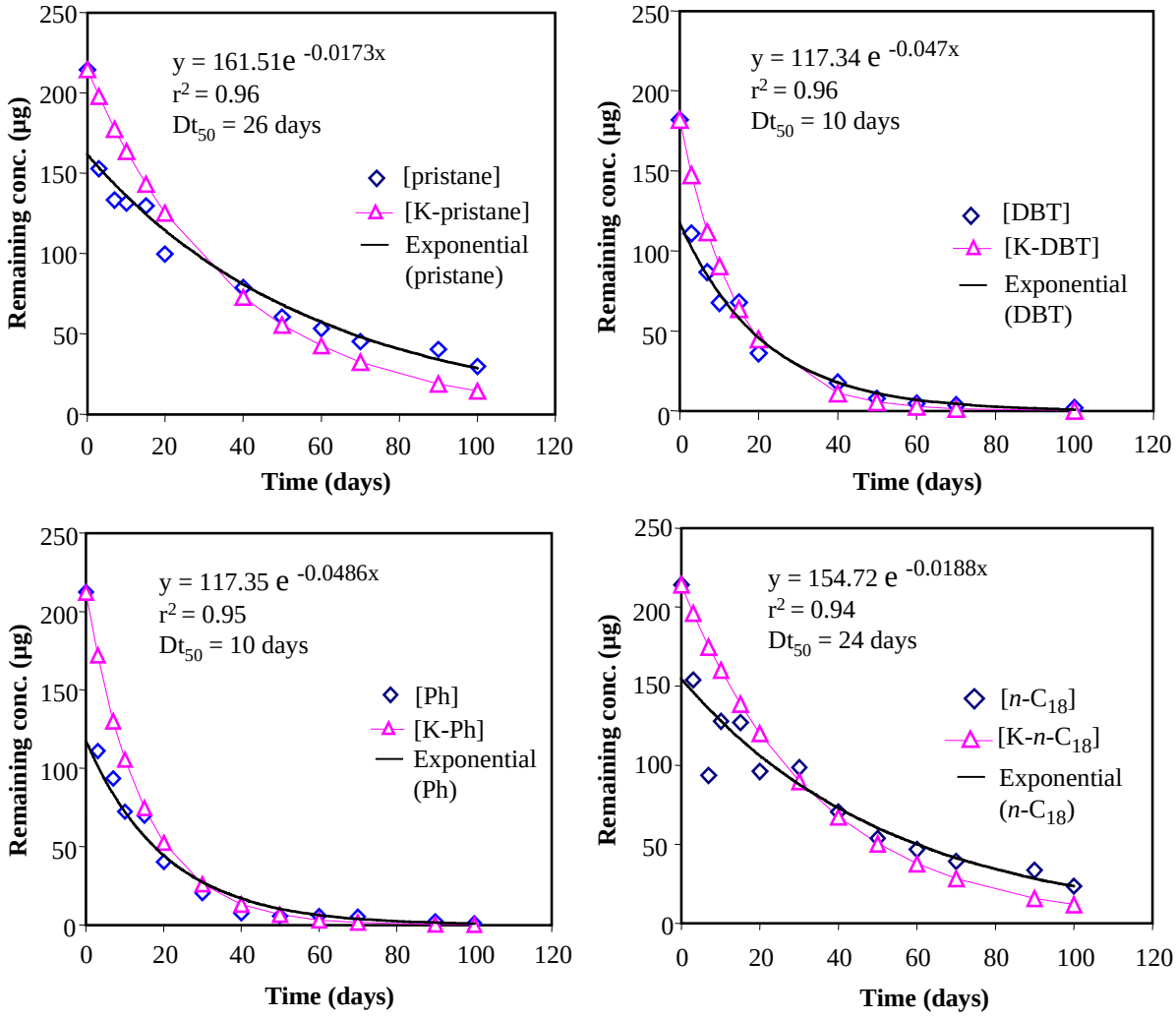


Figure 3.28. Summer degradation of petroleum model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed under field conditions. ◇ represents experimental data, —△— represents linear regression based on the logarithm of the concentration, r^2: correlation coefficient, and Dt_{50}: degradation half-life time.

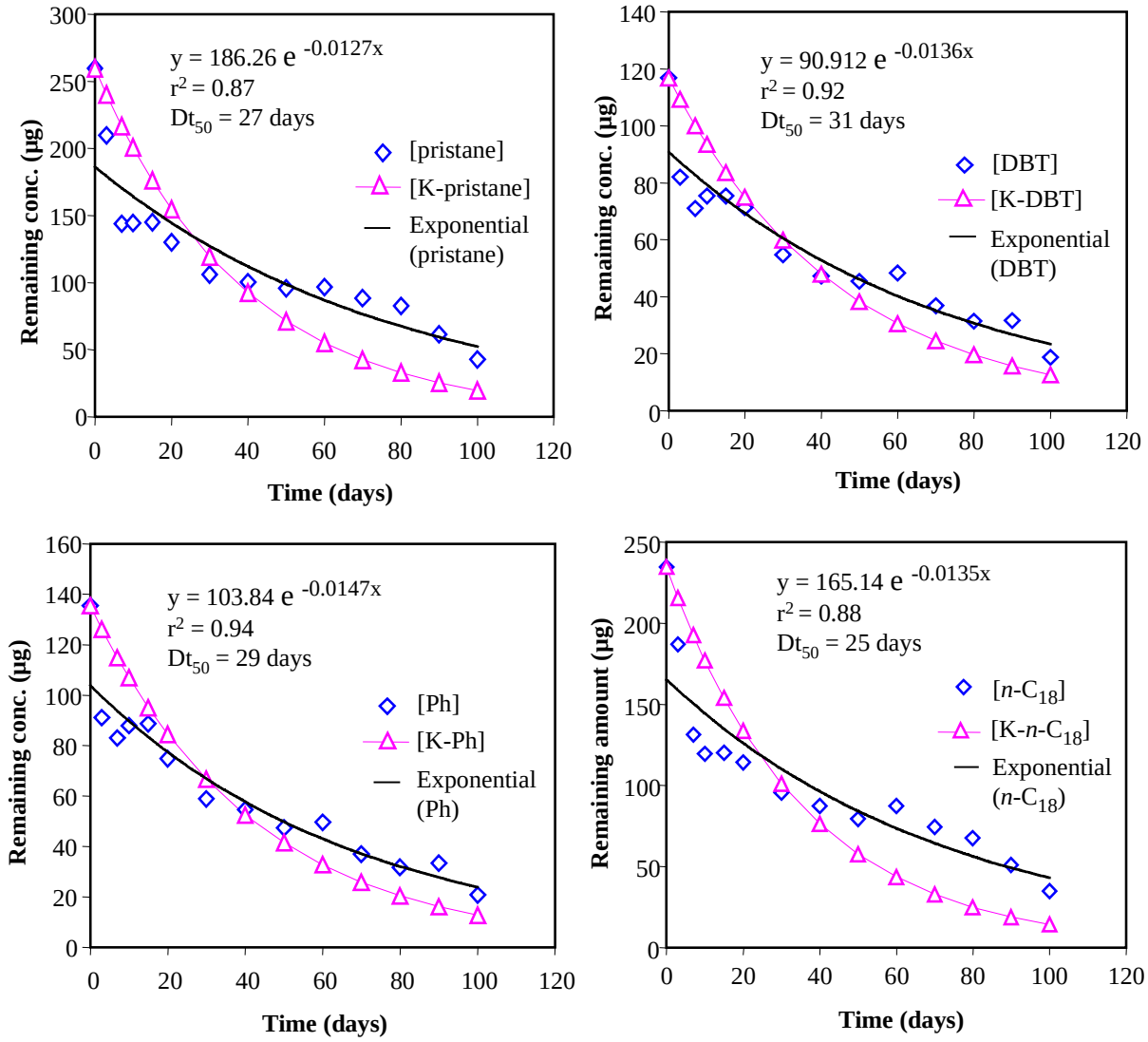


Figure 3.29. Winter degradation of petroleum model compounds in a mesocosm experiment with cyanobacterial mats from Wadi Gaza performed under field conditions in an outdoor experimental pond under the natural climate in Gaza. ◇ represents experimental data, —△— represents linear regression based on the logarithm of the concentration, r^2: correlation coefficient, and Dt_{50}: degradation half-life time.

3.15.6 The use of an organo-clay complex as a carrier for hydrophobic petroleum compounds

Most petroleum constituents are poorly water-soluble limiting their biodegradation in nature. For example, the water solubility of phenanthrene is less than 1.3 mg/L (Pearlman et al. 1984) and that of dibenzothiophene is 1.47 mg/L (Hassett et al., 1980). Therefore, in biodegradation studies, different strategies have been used to make them accessible to microorganisms. This includes either enhancing the solubility of the compounds using

natural or synthetic surfactants and emulsifiers (Barkay et al., 1999; Grimberg et al., 1996) or adsorbing the compounds to solid substrates like clay or resins (Friedrich et al., 2000; Lahlou and Ortega-Calvo, 1999). We selected organo-clay complexes since the inoculum was taken from a surface biofilm of fine-grained Wadi Gaza sediment, and it is likely that the bacteria are adapted to growth on solid surfaces. Furthermore, by the addition of the organo-clay complex we simulated the natural situation in which hydrophobic contaminants are mainly present in adsorbed form. The use of organo-clay complexes in slurry and mesocosm experiments was successful as we could recover more than 75% of the loaded model compounds. This level of recovery was influenced by the experimental procedures such as sampling and chemical analysis. Higher yields were obtained (more than 90%) when the compounds were extracted directly from suspensions of organo-clay complexes (J. Köster, unpublished data). In the dark control without biomass, the constant recovery of more than 75% of the model compounds till the end of the slurry experiment indicates that the organo-clay complex was stable throughout the experiment and that there was no biodegradation (Figure 3.19d). Comparison of light control (Figure 3.21) and dark control (Figure 3.19d) without biomass also demonstrated that the model compounds were not photo-oxidized. The control experiment with autoclaved biomass (Figure 3.19c) showed that adsorption was not significant, even though some loss of the compounds occurred in some cases (Figure 3.19c). This is most likely due to coagulation of clay particles and biomass leading to lower organo-clay complex concentration in the sampled suspension. Consequently, we attribute the disappearance of the model compounds in the presence of living biomass to biodegradation by micro-organisms but not to adsorption or photo-oxidation.

The aromatic compounds were completely degraded in both slurry and mesocosm experiments indicating complete accessibility of the degrading bacteria to these compounds. In contrast, the amount of aliphatic hydrocarbons decreased after 3 days and then remained constant up to 40 days in the slurry experiment. We assume that these compounds were only partially accessible to bacteria. It is likely that they were trapped by strong binding in the clay mineral interlayers, which inhibits desorption or direct biodegradation (Lahlou and Ortega-Calvo, 1999; Theng et al., 1998). This degradation-resistant portion could be extracted with dichloromethane by ultrasonication since this procedure causes a disintegration of aggregates and swelling of the organo-clay complexes.

3.15.7 Conclusions

The results support previous studies of petroleum model compound degradation by microbial mats. Cyanobacterial mats originating from a heavily polluted natural environment of Wadi Gaza effectively degraded both aliphatic (pristane and *n*-octadecane) and aromatic compounds (phenanthrene and dibenzothiophene) in both laboratory and mesocosm experiments. Organo-clay complexes have been successfully applied as a carrier system for the model compounds and allowed the complete degradation of aromatic compounds and most of the aliphatic compounds. In the mesocosm experiments, the re-exposure did not speed up degradation of the model compounds. Degradation of the model compounds in the slurry experiment was faster than in the mesocosm. The degradation rates depend on the type of compound, aromatic compounds were degraded faster than aliphatic hydrocarbons.

3.16 Degradation of herbicides by cyanobacterial mats in slurry experiments

3.16.1 Degradation of 2,4-dichlorophenoxyacetic acid (2,4-D)

Degradation of 2,4-D was studied by its disappearance in the course of the experiments (Figure 3.30). The amount of 2,4-D added to the experimental flasks was 2 mg (corresponding to 20 µg/ml). The extraction of 2,4-D at day 0 yielded 89 to 94% of the theoretical amount. In the control samples without cyanobacterial mats (Figure 3.30), the amounts of 2,4-D indicate that no degradation occurred in the course of the experiments both in the light and in the dark. A lower recovery and a slight decrease of 2,4-D was observed in the light and the dark controls without cyanobacterial mats at day 30 (Figure 3.30). The concentration of 2,4-D applied to the mats decreased steadily in the course of the experiment (Table 3.18).

Table 3.18. Quantities (µg) of residual 2,4-D in a slurry experiment with cyanobacterial mats from Wadi Gaza.

Days	Light [a)]	Dark [a)]	Light [b)]	Dark [b)]	Control [1)]	Control [2)]
0	36.8	36.8	37.5	37.5	35.5	36.8
5	35.7	33.8	34.3	26.7	39.3	39.1
10	30.4	27.8	25.1	23.0	40.7	37.7
12	32.7	28.5	17.0	16.8	39.1	35.9
17	25.3	18.6	12.2	15.9	36.3	36.6
22	18.4	13.1	10.4	12.7	32.2	35.2
27	8.5	6.0	7.8	9.4	30.6	33.1
30	3.9	4.4	5.8	6.9	31.3	32.7

Light: 12 h light/ 12 h darkness; Dark: 24 h darkness; Control [1)]: 12 h light/ 12 h darkness; Control [2)]: 24 h darkness; [a)] Mats from aquarium 2000; [b)] Mats from aquarium 2001.

2,4-D was degraded both in the light and in the dark experiments which were inoculated with cyanobacterial mat material from the aquarium set up in 2000 (hereafter referred to as experiment A) and from the aquarium set up in 2001 (hereafter referred to as experiment B). In the case of experiment A, already after 17 days treatment 31% and 50% of 2,4-D were degraded in the light and in the dark, respectively (Figure 3.30). In the samples of day 22, 64% of 2,4-D were removed in the dark and 50% in the light. The remaining amount of 2,4-D at the end of the experiment was 12% both in the light and the dark (Figure

3.30). In experiment B, 55% of 2,4-D was degraded after 12 days both in the light and the dark. The remaining amount after 22 days was 28% in the light and 34% in the dark (Figure 3.30). At the end of the experiment, more than 80% of 2,4-D were removed both in the light and the dark.

The accumulation and persistence of many chlorinated aromatic compounds in the environment indicate that the ability to use these compounds as carbon or energy source is not widespread among microorganisms. The discovery that many of the degradation systems for chlorinated aromatics are plasmid encoded (Ramos and Timmis, 1987; Ghosal et al., 1985) and that natural genetic exchange can occur in the environment (Trevores and van Elsas, 1989; Genthner et al., 1988) indicates that degradative abilities can evolve and subsequently be transmitted. Although 2,4-D is not considered as recalcitrant as many other chlorinated compounds, its biodegradation in soil and other environments depends on exposure history and acclimation of the indigenous microbial population (Chen and Alexander, 1989; Kim and Maier, 1986).

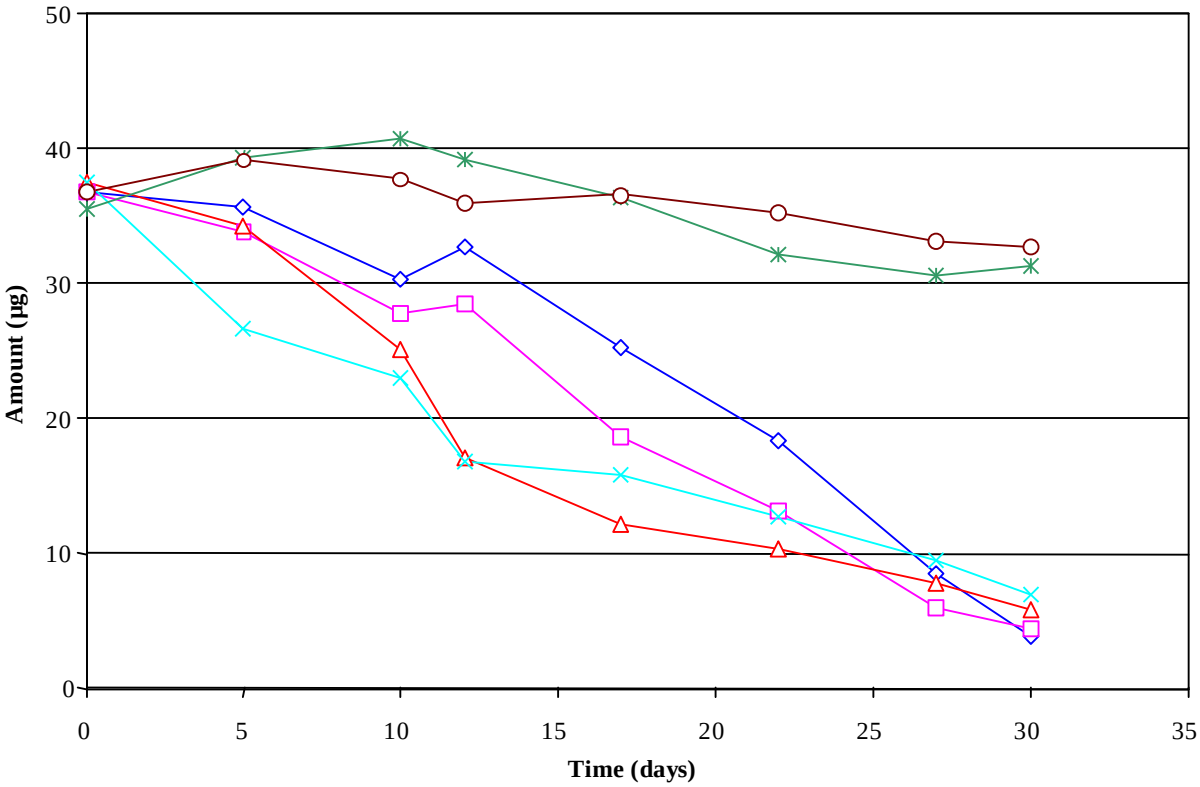


Figure 3.30. Biodegradation of 2,4-D in a slurry experiment with cyanobacterial mats from Wadi Gaza. ◇ light a; □ dark a; △ light b; × dark b; ✳ light control; ○ dark control. A: inoculated from aquarium A; B: inoculated from aquarium B; light: 12 h light/12 h darkness; dark: 24 h darkness.

The constant concentration of 2,4-D in the controls incubated in the light and in the dark (Figure 3.30) indicates that 2,4-D was not adsorbed in significant amounts to the glass walls of the flasks. This result is in agreement with intact cyanobacterial mat experiments in an aquarium, in which 4.6% of 2,4-D was lost (Grötzschel, 2001). In addition, no sorption of 2,4-D was observed in column experiments using aquifer material (Tuxen et al., 2000). The first report on direct photolysis of 2,4-D was cited in 1966 (Crosby and Tutass 1966). It follows first-order kinetics with respect to the absorbed light but is independent on the 2,4-D concentration (Cabrera et al., 1997). Grötzschel (2001) found that a small amount of 2,4-D was degraded photochemically in the experiments with intact mats. In the present study photodegradation is excluded since the control treatment without mat material did not show the concentration changes that were observed in the treatment with mat organisms. The same decrease in 2,4-D (12%) had occurred in both light and dark controls at the end of the experiment. This indicates that the loss of 2,4-D was due to adhesion phenomena not to photolysis. Metabolites were not expected to be observed in the present study since the two main photochemical reaction pathways with the intermediates 2,4-dichlorophenol and chlorohydroquinone end in total mineralization of the compounds (Alfano et al., 2001).

Degradation of 2,4-D was slightly faster in the dark than in the light in the first two weeks of experiment A. After 10 days of a slow rate of disappearance, the degradation rate increased both under light and dark conditions. At the end of the experiment the same amount (88%) of 2,4-D was removed in both the light and the dark. This suggests that the microbial mat is not homogeneous and a kind of adaptation had occurred.

In experiment B, nearly the same degradation level of 2,4-D was observed in the course of the experiment in both the light and the dark. There are no significant differences between the experiment inoculated from aquarium A and the experiment inoculated from aquarium B in the removed amounts of 2,4-D at the end of the experiment. Grötzschel (2001) reported that 35% of 2,4-D was degraded in laboratory experiments with intact cyanobacterial mats within 13 days. Three bacterial isolates (*Xanthomonas maltophilia* SB5, *Pseudomonas sp*, SB9 and *Rhodococcus globerulus* AS(2)) degraded 91% of 2,4-D in non-contaminated soil within 28 days (Mcghee and Burns, 1995). This is in agreement with the result of the present study, since more than 85% of 2,4-D were removed within 30 days.

3.16.2 Degradation kinetics

According to regression analysis, the data for the degradation of 2,4-D in experiment A fits zero-order kinetics as described by Segel (1976). The equation is $\ln C_t = \ln C_0 - Kt$, where C_0 represents the initial concentration, C_t represents the concentration at time t, t represents the time in days and K is the decay rate constant. The larger the value of K, the faster is the degradation process. In the case of experiment B, first-order kinetics has been observed. First-order rate constants (K), correlation coefficient (r^2) and the time required for 50% degradation of the tested compound (Dt_{50}) calculated for the degraded 2,4-D are presented in Table 3.19.

Table 3.19. First-order degradation rate constant, correlation coefficient and degradation half-life time of 2,4-D in a slurry experiments with cyanobacterial mats.

Degradation	**Linear**		**Exponential**	
Conditions	**light A**	**dark A**	**light B**	**dark B**
K	1.14	1.17	0.064	0.052
r^2	0.93	0.98	0.97	0.98
Dt_{50}	18	17	13	12

K = degradation rate constant, r^2 = correlation coefficient and Dt_{50} = degradation half-life time (days), A = inoculated from aquarium 2000, B = inoculated from aquarium 2001, light = 12 h light/12 h darkness and dark = 24 darkness.

In experiment A, it can be seen that the degradation pattern follows a linear trend, and an exponential trend is seen in experiment B (Figure 3.30). The correlation coefficients (r^2) are larger than 0.93 for the linear degradation and larger than 0.97 for the exponential degradation (Table 3.19 and Figure 3.30). Comparing the Dt_{50} values (time required for 50% decay of 2,4-D), there is no significant difference for the 2,4-D between light and dark conditions in both experiments A or B. There is a significant difference, however, in Dt_{50} for the 2,4-D between experiments A and B (Table 3.19). This may indicate that the microbial mats from aquarium 2001 were enriched in species capable of degrading 2,4-D whereas the microbial mats from aquarium 2000 were not.

In the laboratory experiment with intact cyanobacterial mat from Guerrero Negro (Mexico), the degradation of 2,4-D was apparently more effective under oxic than under anoxic conditions (Grötzschel, 2001). Faster degradation of 2,4-D under oxic conditions in muck soil with a Dt_{50} of 3.4 days but with 35.9 days in a sandy loam was reported by Cheah et al. (1998). The Dt_{50} of 2,4-D in the experiment with intact cyanobacterial mats was 6.7

days (Grötzschel, 2001) and in fine sand 23 days (Thompson et al., 1984). Compared to these literature data, the rate of degradation of 2,4-D in the present experiment is intermediate, with a Dt_{50} of 12 and 17 days in experiments A and B, respectively.

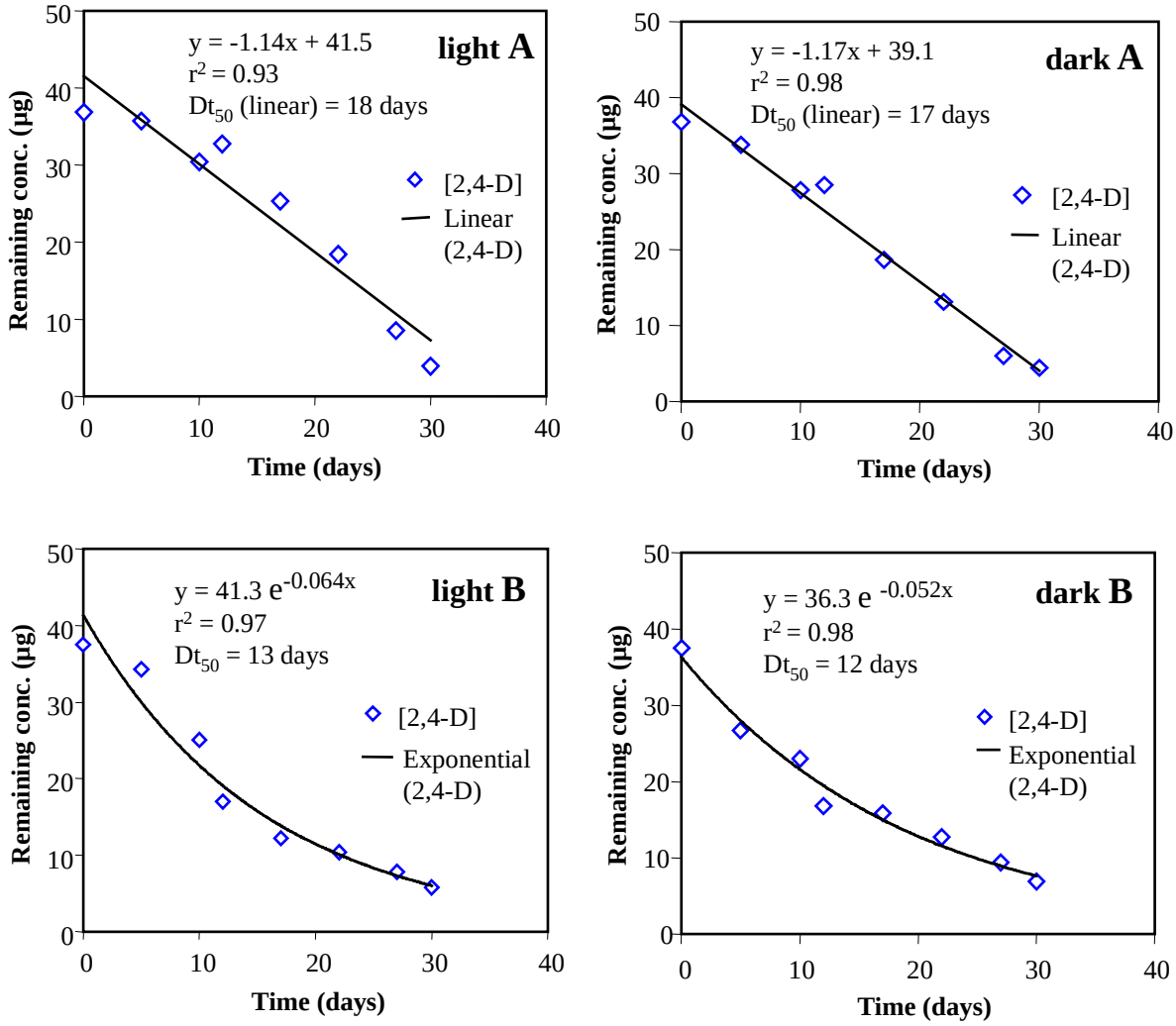


Figure 3.31. Linear and exponential biodegradation of 2,4-D in a slurry experiments with cyanobacterial mats from Wadi Gaza. Light: 12 h light/12 h darkness; Dark: 24 darkness; A: inoculated with mats from the aquarium set up in 2000; B: inoculated with mats from the aquarium set up in 2001.

3.16.3 *Degradation of terbutryn*

The bioremediation assay with terbutryn [2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-*s*-triazine] was performed under laboratory conditions in a slurry experiment. The amount of terbutryn added to the experimental flasks was 0.36 mg (corresponding to 3 µg/ml). Extraction of terbutryn at day 0 yielded 87 to 89% of the theoretical amount of the terbutryn. In the control samples, i.e. terbutryn without cyanobacterial mat material (Figure 3.32), the amounts of terbutryn indicate that no degradation occurred in the course of the experiments. A lower recovery and a slight decrease of terbutryn was found in the control after 40 days; this pattern remained constant until the end of the experiment (Figure 3.32). The concentration of terbutryn applied to the mat slurry decreased steadily (Table 3.20).

Table 3.20. Quantities (µg) of residual terbutryn in a slurry experiment with cyanobacterial mats from Wadi Gaza.

Time (days)	Light	Dark	Control
0	13.4	13.0	13.0
10	12.1	11.2	13.3
30	10.0	8.0	11.8
40	9.30	7.6	12.1
60	5.9	5.9	12.4
80	3.8	4.7	12.0

Light: 12 h light/12 h darkness; Dark: 24 darkness; Control: 12 h light/12 h darkness (no mats).

Degradation of terbutryn was studied by its disappearance in the course of the experiment (Figure 3.32). Terbutryn was degraded in both light and dark experiments which were inoculated with cyanobacterial mats from Wadi Gaza. Already after 40 days of treatment 31% and 42% of the terbutryn was degraded in the preparations incubated in the light and in the dark, respectively (Figure 3.32). In the samples of day 60, 55% of the terbutryn was removed under both dark and light conditions. At the end of the experiment the remaining amounts of the terbutryn were 28% of the starting amounts in the preparation incubated in the light and 36% in dark (Figure 3.32). The nearly constant concentration of terbutryn in the control incubated in the light (Figure 3.32) indicates that terbutryn was not volatilized in significant amounts. In water, terbutryn is not volatile (U.S. Department of Health and Human Services, 1993). Volatilization losses of terbutryn from 1 to 6% in soils were reported by Tabernero et al. (2000).

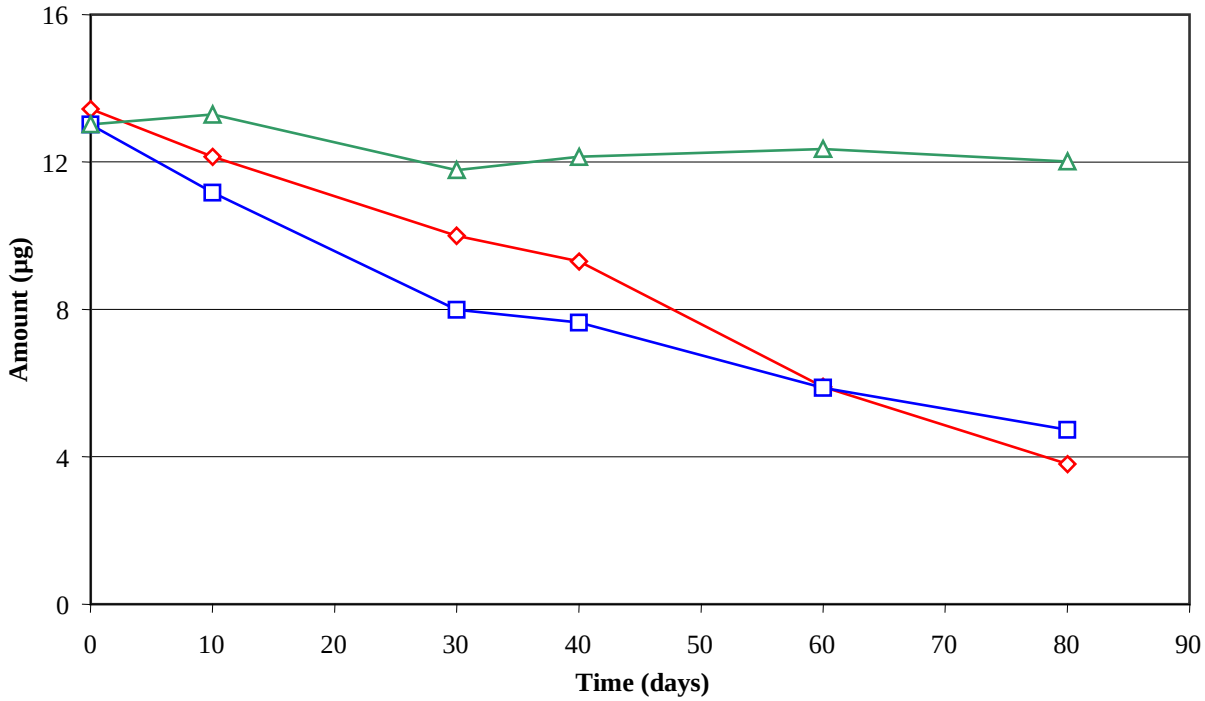


Figure 3.32. Biodegradation of terbutryn in a slurry experiment with cyanobacterial mats from Wadi Gaza. ◇ 12 h light/12 h darkness; □ 24 h darkness △ control 12 h light/12 h darkness.

Degradation of the terbutryn applied to the mats started immediately after addition of the herbicide. It slowed down in the dark and accelerated in the light in the course of the experiment (Figure 3.32). In the first 40 days, degradation of terbutryn was slightly faster in the dark than in the light. After 40-60 days, the removed amount of terbutryn in the light was twice the amount removed in the dark. This may indicate that the diversity of the species in the microbial mats were different between the light and dark experiments and that a kind of adaptation had occurred. Photodegradation of triazine-type herbicides like terbutryn was reported by Lanyi and Dinya (2002, 2003). At the end of the experiment, the difference of 8% in the removed amounts of terbutryn between the light and the dark may be due to photodegradation because 7.7% of terbutryn was lost in the control incubated in the light. The indigenous microorganisms inhabiting the highly polluted Wadi Gaza site from which the cyanobacterial mats used in the present study were taken appear to have a strong potential to effectively degrade herbicides.

There are relatively few published reports on the degradation of terbutryn in the environment, but hydroxytriazines, N-deethylated triazines and their photooxidation products have been reported as terbutryn decomposition products (Burkhard and Guth, 1976). Muir (1980) found terbutryn and several of its degradation products, N-deethylated terbutryn,

hydroxyterbutryn, N-deethylated hydroxyterbutryn, 2-(*tert*-butylamino)-4-(ethylamino)-*s*-triazine (EBT), and 2-(*tert*-butylamino)-4-amino-*s*-triazine (ABT), in water samples from a pond treated with terbutryn. All the compounds were found in the water except ABT and EBT which were also found in the sediment.

3.16.4 *Degradation kinetics*

The biodegradation of terbutryn fits zero-order kinetics (linear degradation) in the preparation incubated in the light and first-order kinetics (exponential degradation) in the preparation incubated in the dark (Figure 3.33). The correlation coefficient values (r^2) are 0.99 for the linear and the exponential degradation. Comparing the Dt_{50} values of terbutryn, their is no significant difference between the light (56 days) and the dark conditions (49 days).

Degradation rates of triazine-type herbicide like terbutryn depend on the environmental conditions. The Dt_{50} of terbutryn in soil is 14-28 days (Unwin Brothers Ltd., 1994). Dt_{50} of 240 and 180 days have been reported for degradation of terbutryn in pond and river sediments, respectively, by Muir and Yarechewski (1982). The observed Dt_{50} of 49 and 56 days in the present experiment may indicate that fast degradation of terbutryn occurred by the Wadi Gaza cyanobacterial mat organisms.

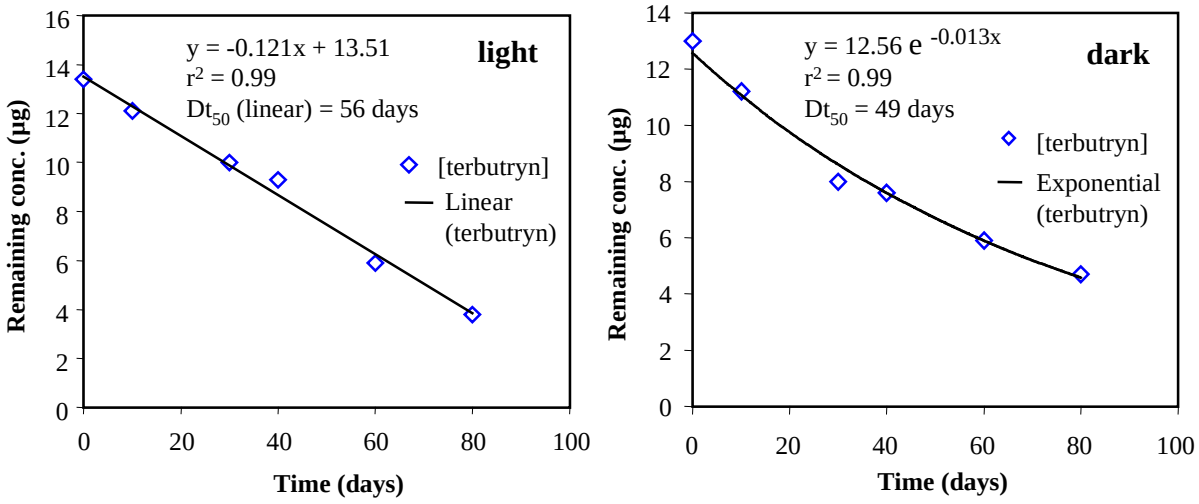


Figure 3.33. Linear and exponential biodegradation of terbutryn in slurry experiments with cyanobacterial mats from Wadi Gaza. Light: 12 h light/12 h darkness; Dark: 24 darkness.

3.16.5 Conclusions

The results of the slurry experiments with terbutryn and Wadi Gaza cyanobacterial mat material support previous studies of herbicide degradation by microbial communities. Cyanobacterial mats originating from a heavily polluted natural environment of Wadi Gaza effectively degraded both 2,4-D and terbutryn in laboratory experiments. Their is no significant difference in half-life time of 2,4-D between light and dark conditions. This indicates that degradation of 2,4-D by microorganisms is not light dependent. The difference in the degradation kinetics between experiment A which was inoculated with mats from the aquarium set up in 2000 and B which was inoculated with mats from the aquarium set up in 2001 suggests that the diversity of unknown microorganisms which contribute to biodegradation is different. It is, however, needed to analyze the mats by molecular biological methods in order to explain the differences. The results of the present study indicate that the examined mats had a high capacity for herbicide degradation.

4. Summary

The overall aims of this thesis work were:

1) to study the organic geochemistry of Wadi Gaza sediments and their level of pollution, particularly with petroleum and petroleum derivatives. For this purpose surface sediments samples were collected from different sites along Wadi Gaza (Gaza Strip, Palestine) and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) techniques.

2) to investigate the ability of naturally occurring cyanobacterial mats inhabiting the heavily polluted natural environment of Wadi Gaza to degrade Egyptian crude oil, petroleum model compounds (*n*-octadecane, pristane, phenanthrene and dibenzothiophene) and herbicides in small-scale laboratory experiments and partly in mesocosm experiments under the natural climatic conditions of the Gaza Strip. The petroleum model compounds represent important groups of petroleum constituents (straight-chain alkanes, branched alkanes, aromatic hydrocarbons and organo-sulfur compounds).

The organic matter composition of extractable lipids in the sediments indicates that Wadi Gaza receives a moderate supply of anthropogenic pollution. The composition of aliphatic hydrocarbons in the Wadi Gaza sediments were identified to derive from petroleum sources. The presence of acyclic isoprenoid alkanes (pristane and phytane), the presence of an unresolved complex mixture (UCM), the UCM/*n*-alkane ratio, the carbon preference index close to 1 and the presence of petroleum biomarkers such as steranes and hopanes indicate the presence of petroleum and petroleum derivatives. The biomarker parameters based on hopanes and steranes reveal significant differences between the sediment samples which suggests that there are multiple sources of oil or refinery products. In the aromatic hydrocarbon fraction a contribution of petroleum origin is indicated by the presence of phenanthrene and its alkylated homologues as well as that of dibenzothiophene and its alkylated homologues. The ratio of methylphenanthrenes to phenanthrene shows that the sediments are dominated by fossil fuel derived phenanthrenes and not by combustion residues. The methyldibenzothiophene index (MDI) of Wadi Gaza sediments confirms the input of crude oil and petroleum derivatives. Compounds derived from terrestrial sources (higher plants), such as long-chain *n*-alkanes, *n*-fatty acids and *n*-alcohols, and marine-derived hydrocarbons were also present.

The observation of microbial degradation of petroleum hydrocarbons in Wadi Gaza sediments stimulated studying the biodegradation of organic pollutants by Wadi Gaza cyanobacterial mats.

In the experiment with crude oil, the mats efficiently degraded *n*-alkanes and the isoprenoids pristane and phytane both in the light and in the dark. The biodegradation rates for *n*-alkanes in crude oils often tend to decrease as chain length increases (Holder et al., 1999; Pond et al., 2001). In the experiment with Gaza mats, short-chain *n*-alkanes from C_{14} to C_{20} and the long-chain *n*-alkanes from C_{28} to C_{35} were degraded faster than those of medium chain length with 21 to 27 carbon atoms. This suggests that the degradation pattern is due to the specific composition of the microbial community.

Pristane and phytane were more resistant to biodegradation than normal alkanes, as also observed by Atlas (1981) and Basseres and Ladousse (1993). Since in the present study 94% and 88% of pristane and phytane, respectively, were degraded in 60 days, we conclude that the degradation rates of these isoprenoidals alkanes were high.

In the petroleum model compounds experiment, Wadi Gaza cyanobacterial mats efficiently degraded the aromatic compounds phenanthrene and dibenzothiophene completely in 7 days both in the light and in the dark in the laboratory experiments. The aliphatic compounds *n*-octadecane and pristane were degraded to 27% and 36%, respectively, within 7 days but no further degradation occurred till 40 days.

To elucidate the degradation potential and degradation kinetics of the cyanobacterial mats for the same petroleum compounds in large scale a follow-up experiment was designed based on the results for the small-scale experiments. For this purpose two cylindrical shaped concrete ponds were constructed at the experimental station of EPRI in Gaza to allow mesocosm experiments. The large-scale experiments with intact cyanobacterial mats were carried out under field conditions in an outdoor experimental pond under the natural climate in Gaza.

In the large-scale experiments performed in summer, 96% of the aromatic substances dibenzothiophene and phenanthrene were degraded in 40 days. After 70 days these compounds were completely removed. The aliphatic hydrocarbons pristane and *n*-octadecane were degraded to 21% and 18%, respectively, within 70 days. At the end of the experiment the remaining amounts of these compounds were below 15% of the starting material.

A second experiment was conducted in order to investigate the effect of repeated exposure to contaminants and the performance of the mats during winter time. The same amount of contaminants was added to the same pond after the 100 days period of the summer experiment. Sixty percent of the dibenzothiophene and phenanthrene were degraded in 40 days. At the end of the experiment, 85% of the phenanthrene, dibenzothiophene, *n*-octadecane and pristane were degraded. An apparently non-degradable residual concentration of aliphatic hydrocarbons at the 20% level was already observed in all earlier degradation experiments with organo-clay complexes due to incomplete bioavailability (Safi et al., 2001; Abed et al., 2002; Safi et al., 2003).

A significant difference in the growth of the mats between the treatment and the control were observed during the experiment. There is also a significant difference in the degradation of the aromatic compounds between summer and winter. In the samples of day 40, 96% and 60% of these compounds were degraded in summer and winter, respectively. After 40 days, the degradation of the aromatic compounds slowed down in both summer and winter experiments. This indicates that the contaminants had reduced the activity of certain microorganisms responsible for the degradation of the aromatic compounds. Pollution with these compounds inhibits photosynthesis and growth, reduces enzyme activity and microbial biomass and induces changes in the cyanobacterial species composition (Megharaj et al., 2000).

Comparing the Dt_{50} value (time required for degradation of 50% of the parent compound) shows a short degradation time for phenanthrene and dibenzothiophene (10 days) in the summer experiment. In the winter experiment, shorter degradation times were observed for the aliphatic compounds pristane and *n*-octadecane, i.e. 27 and 25 days, respectively. Degradation of the model compounds in the laboratory experiment was faster than in the mesocosm.

The re-exposure of the mats to the contaminants did not speed up degradation of the four model compounds. Statistical analysis showed significant differences in Dt_{50} for the aromatic compounds between summer and winter. These results suggest that light and temperature enhance the degradation activity of the cyanobacterial mats especially for the aromatic compounds as observed in the summer experiment.

To increase the availability of selected hydrophobic petroleum compounds to submersed cyanobacterial mats, organo-clay complexes have been successfully applied as a carrier system for crude oil and model compounds and allowed the complete degradation of aromatic compounds and most of the aliphatic compounds. The degradation rates depend on the type of compound, aromatic compounds were degraded faster than aliphatic hydrocarbons.

Cyanobacterial mat from Wadi Gaza incubated at 12 h light/12 h darkness and 24 h darkness degraded more than 80% of 2,4-Dichlorophenoxyacetic acid (2,4-D) within 30 days. The major removal process was microbial mineralization in the light and in the darkness. Photodegradation is excluded since the control treatment without mat material did not show the concentration changes that were observed in the treatment with mat. Comparing the Dt_{50} values their is no significant difference for the 2,4-D degradation between light and dark conditions in both experiments inoculated with microbial mats. This indicates that degradation of 2,4-D by microorganisms is not light dependent. Their is a significant difference, however, in Dt_{50} for the 2,4-D between experiments A and B. This indicates that the microbial mats from aquarium 2001 were enriched in species capable of degrading 2,4-D whereas the microbial mats from aquarium 2000 were not. It is, however, needed to analyze the mats by molecular biological methods in order to explain the differences.

Terbutryn was degraded to 28% and 36% of its original concentration in the light and in the darkness, respectively, within 80 days. Terbutryn was reported to degrade slowly, with half-lives time of 240 and 180 days in pond and river sediment, respectively (Muir and Yarechewski, 1982). The observed half-lives time of 56 and 49 days in the light and in the dark, respectively, in our experiment indicate that fast degradation of terbutryn occurred by the Wadi Gaza cyanobacterial mat organisms.

We conclude that the sediments from Wadi Gaza are heavily polluted with crude oil and petroleum derivatives. Wadi Gaza cyanobacterial mats represent a robust and efficient ecosystem for the degradation of petroleum derivatives and herbicides.

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6. Appendix

6.1 Tables

Table A1 Percent of degradation of model compounds in the mesocosm experiment performed in summer and winter with cyanobacterial mats from Wadi Gaza.

	Time days	Site 1				Site 2				Site 3			
		Pr	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**
Summer	7	28	38	42	28	36	55	59	36	54	68	70	53
	40	55	94	94	57	76	99	99	80	58	94	95	65
	70	80	96	97	82	88	99	99	90	67	96	96	71
	100	90	100	100	93	89	100	100	91	78	100	99	82
Winter	7	61	51	55	58	2	38	31	1	55	26	26	58
	40	58	64	66	56	45	62	56	46	74	56	56	77
	70	54	69	77	56	54	69	72	59	78	66	68	82
	100	87	85	87	86	78	89	88	81	84	76	78	87

Pr: pristane; DBT: dibenzothiophene; Phen: phenanthrene and *n*-C_{18}: *n*-octadecane
Summer experiment: performed from 22nd July till 30th October 2001
Winter experiment: performed from 30th October 2001 till 8th February 2002

Table A2 Average percentage of degradation of model compounds for three sites in the mesocosm experiment performed in summer and winter (2001-2002) with cyanobacterial mats from Wadi Gaza.

Time days	Summer				Winter			
	Pr	**DBT**	**Phen**	***n*-C_{18}**	**Pr**	**DBT**	**Phen**	***n*-C_{18}**
7	38	52	56	38	45	39	39	44
40	63	96	96	67	62	60	60	63
70	79	100	100	82	66	69	73	68
100	86	100	100	89	84	84	85	85

Pr: pristane; DBT: dibenzothiophene; Phen: phenanthrene and *n*-C_{18}: *n*-octadecane

Table A3 Water depth, temperature, salinity and pH value during degradation of model compounds in the mesocosm experiments performed in summer with cyanobacterial mats from Wadi Gaza.

Summer experiment					Aquarium control			
Time days	**Depth (cm)**	**Temp. (°C)**	**Salinity (%)**	**pH**	**Depth (cm)**	**Temp. (°C)**	**Salinity (%)**	**pH**
0	30	35	4.5	8.5	30	31	4.3	8.5
3	30	35	4.9	8.5	30	32	4.4	8.3
7	28	30	4.1	8.6	28	35	4.3	8.3
10	28	35	4.2	8.6	25	35	5.5	8.3
15	25	33	4.6	8.7	20	35	7.2	8.3
20	23	34	5.0	8.7	15	42	10.0	8.7
30	17	33	7.1	9.1	25	40	6.4	9.9
40	24	34	5.8	8.3	30	33	4.4	7.6
50	30	35	4.6	8.9	30	29	4.3	7.4
60	30	35	4.6	9.5	25	25	5.1	8.4
70	32	30	4.1	8.5	30	28	5.0	8.4
80	30	33	4.6	8.2	30	28	4.9	8.1
90	30	30	4.5	8.3	30	28	5.1	8.0
100	30	29	4.5	8.4	28	28	5.3	8.0

Summer experiment: performed from 22nd July till 30th October 2001

Table A4 Water depth, temperature, salinity and pH value during degradation of model compounds in the mesocosm experiments performed in winter with cyanobacterial mats from Wadi Gaza.

Winter experiment					Aquarium control			
Time days	**Depth (cm)**	**Temp. (°C)**	**Salinity (%)**	**pH**	**Depth (cm)**	**Temp. (°C)**	**Salinity (%)**	**pH**
0	30	30	4.4	7.0	30	16	4.5	6.6
3	30	25	4.2	5.7	32	16	4.5	6.6
7	30	29	4.3	6.8	32	15	4.4	6.6
10	30	25	4.4	6.8	31	16	4.5	6.6
15	30	23	4.6	7.0	33	16	4.6	7.2
20	30	17	5.1	6.9	32	15	4.4	7.3
30	30	22	4.8	6.0	40	11	3.4	7.2
40	45	18	3.0	6.3	40	14	3.7	6.1
50	48	15	3.0	7.3	40	14	3.8	6.3
60	48	15	3.5	7.5	40	13	3.9	6.7
70	49	11	2.5	7.5	40	15	3.8	6.2
80	56	14	3.0	6.5	38	17	3.9	6.5
90	57	13	2.5	7.1	34	17	4.2	7.0
100	57	18	2.4	6.4	34	20	4.2	6.4

Winter experiment: performed from 30th October 2001 till 8th February 2002

Table A5 Water depth, temperature, salinity and pH value for the control section without model compounds during degradation of model compounds in the mesocosm experiments performed in summer and winter with cyanobacterial mats from Wadi Gaza in Gaza.

Summer experiment					Winter experiment			
Time days	**Depth (cm)**	**Temp. (°C)**	**Salinity (%)**	**pH**	**Depth (cm)**	**Temp. (°C)**	**Salinity (%)**	**pH**
0	30	35	4.3	8.5	30	30	4.7	7.1
3	30	35	4.4	8.7	30	25	4.7	7.1
7	28	30	4.2	8.8	30	29	4.8	7.2
10	28	35	4.5	8.7	30	25	4.8	7.0
15	25	33	5.1	8.8	30	23	4.7	7.0
20	23	34	5.4	8.8	30	17	5.2	7.0
30	17	35	6.2	8.9	30	22	4.9	6.3
40	24	35	5.5	9.0	45	18	2.9	7.0
50	30	35	4.8	9.1	48	15	3.7	6.7
60	30	35	4.9	8.0	48	15	3.6	7.1
70	32	30	4.2	7.5	49	11	3.3	7.0
80	30	33	5.1	8.5	56	14	2.5	7.4
90	30	31	5.0	8.4	57	13	3.1	7.4
100	30	29	5.0	8.2	57	18	2.6	7.8

Summer experiment: performed from 22nd July till 30th October 2001
Winter experiment: performed from 30th October 2001 till 8th February 2002

6.2 Figures

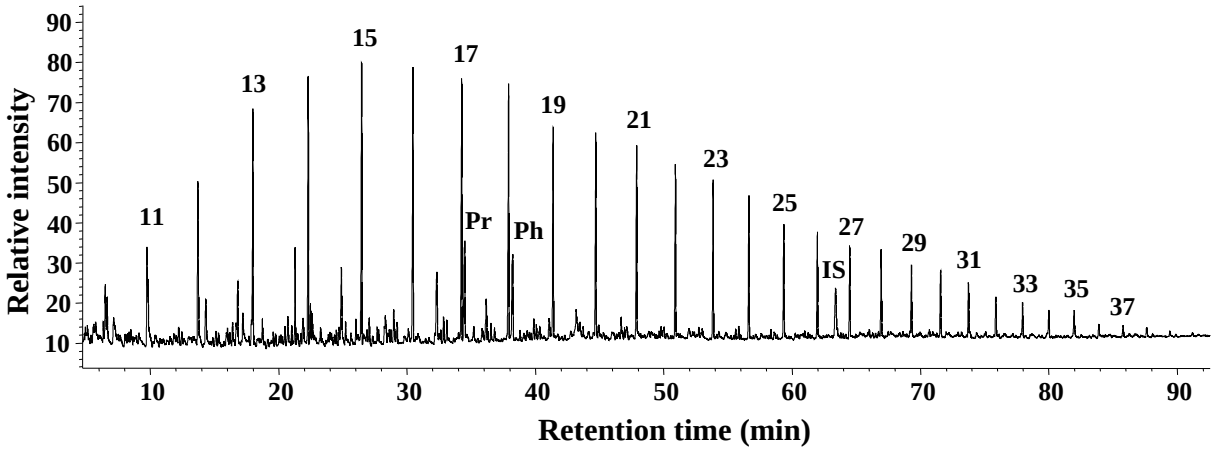


Figure A1 Representative gas chromatogram of stabilized Egyptian crude oil which was loaded on organo-clay and used in the slurry experiments with Wadi Gaza cyanobacterial mats. Numbers indicate carbon numbers of *n*-alkanes; IS = internal standard; Pr = pristane; Ph = phytane.

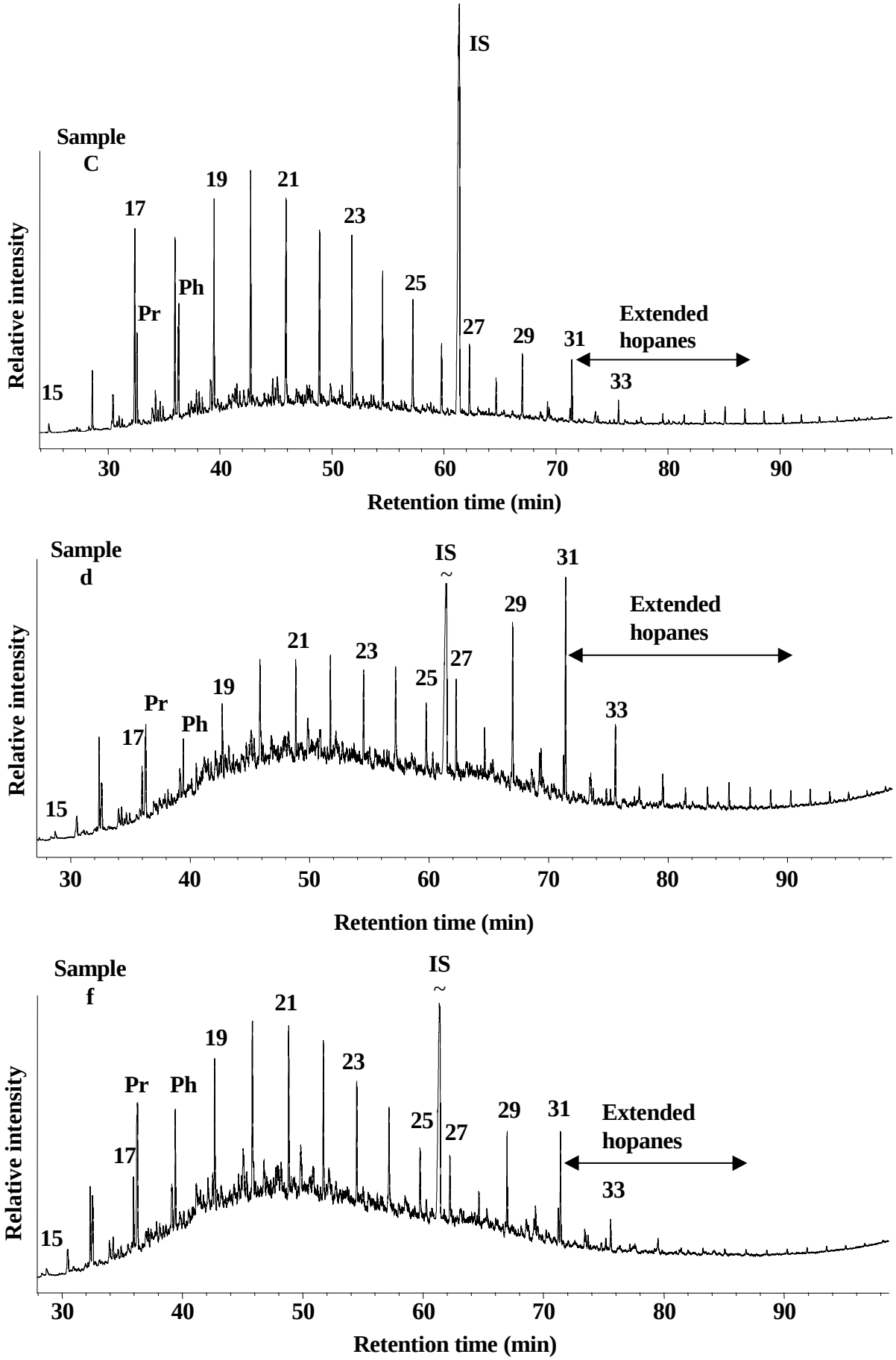
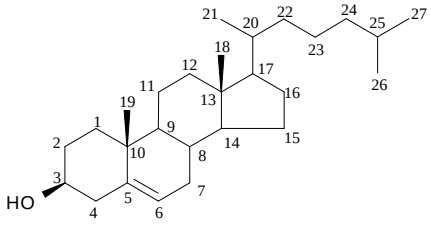
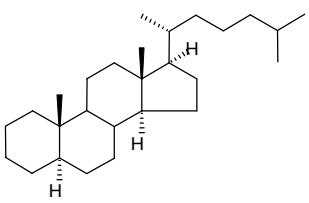


Figure A2 Representative gas chromatograms of three aliphatic hydrocarbon fractions extracted from Wadi Gaza sediments (sample c, d and f). Numbers indicate carbon numbers of *n*-alkanes; ~ = reduced peak; IS = internal standard; Pr = pristane; Ph = phytane.

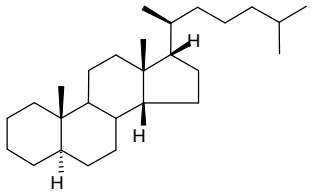
6.3 *Chemical structures of some biomarkers*



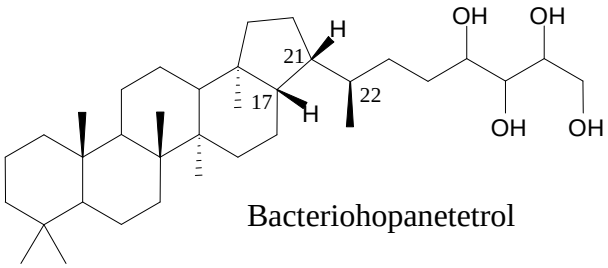
Cholesterol
(Cholest-5-en-3β-ol)



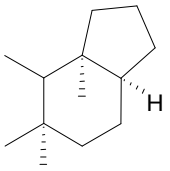
5α,14α,17α,20*R*-
Cholestane



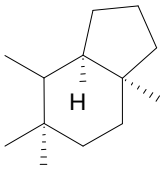
5α,14β,17β,20*S*-
Cholestane



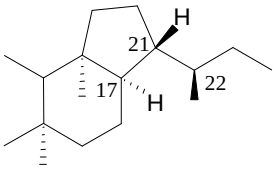
Bacteriohopanetetrol



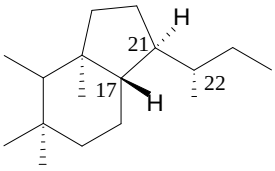
22,29,30-*trinor*-
17α-Hopane (Tm)



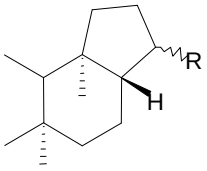
22,29,30-*trinor-neo*
18α-Hopane (Ts)



17α,21β,22R-
homo-Hopane

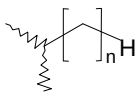


17β,21α,22S-
homo-Hopane



R =  30-*nor*-17α-Hopane

R =  17α-Hopane

R = 

n = 2 *homo*-17α-Hopane
n = 3 *dihomo*-17α-Hopane
n = 4 *trihomo*-17α-Hopane
n = 5 *tetrakishomo*-17α-Hopane
n = 6 *pentakishomo*-17α-Hopane

6.4 Photographs

Figure A3 **A**: Sampling sediments from the western part of Wadi Gaza; **B**: Sampling cyanobacterial mats which developed in polluted environment ; **C**: Showing a side of Wadi Gaza totally dry in summer (August, 2001).

Curriculum Vita

Name:	Nimer Mohamed Deeb Safi
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09/1973-05/1979	Elementary School B for Refugees in Jabalia Camp, Gaza Strip, Palestine
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10/1985-02/1994	B.Sc. Chemistry, Faculty of Science, The Islamic University of Gaza, Gaza Strip, Palestine (The University was closed due to the political situation from 1988 until 1991)
10/1996-10/1997	Master of Engineering in Environmental Science and Technology (Environmental Management), International Institute for Infrastructural, Hydraulic and Environmental Engineering, (IHE), Delft, the Netherlands
11/1997-06/1998	Scientific Researcher at Environmental Protection and Research Institute (EPRI), Gaza, Gaza Strip, Palestine
07/1998-12/1999	Scientific Researcher at (EPRI), Gaza, Gaza Strip, Palestine in project “Bioremediation of Marine Pollutants by Marine Cyanobacterial Mats”
01/2000-06/2000	Ph.D. student at the University of Oldenburg.
07/2000	Ph.D. student and Scientific Researcher at Institute of Chemistry and Biology of the Marine Environment (ICBM), Organic Geochemistry group, in the project “Bioremediation of Marine Pollutants by Marine Cyanobacterial Mats”

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig angefertigt habe, keine anderen als die angegebenen Quellen verwendet habe und dass die Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vorliegt oder vorgelegen hat.

Oldenburg, den 03. 02. 2004