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VARIABILITY IN GENOMIC PATTERNS OF BACTERIAL COMMUNITIES RELATED TO TARMATS AND QUANTIFICATION OF HYDROCARBON PRESENT IN THE TARMATS DEPOSITED ALONG THE QATAR COAST

BY

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ABSTRACT

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Title: Variability in Genomic Patterns of Bacterial Communities Related to Tarmats and Quantification of Hydrocarbon Present in the Tarmats Deposited along the Qatar Coast

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Qatar has undergone a rapid transition from a fishing and small agriculture to an economy focused on oil and gas since 1960. Owing to the comparatively large level of oil and gas production, the region has become severely contaminated by oil pollution including tar residues on the coast. Such chemical and biologically transformed oil residues are particularly important because they are expected to degrade gradually. In the present study, tarmat samples collected from 16 locations along the Qatar coast were subject to chemical and metagenomics analyses. The study includes investigation of bacterial community by *16S rRNA* based identification of isolates, followed by Illumina sequencing and metagenomic analysis along with determination of TPH and PAH concentrations by GC and ATR-FTIR analyses. GC-MS analyses confirmed the presence of PAHs in tarmat samples, with the highest level of Benzo(g,h,i)perylene, collected from Al Arish (13.893ppb), Abu Samra (11.973ppb) and Ras Laffan (11.530). High molecular weight (HMW) PAHs dominated in the total PAH composition, and low molecular weight (LMW) PAHs such as fluorene and phenanthrene were not detected in any of the studied tarmat samples. ATR-FTIR absorption spectra for tarmat samples showed notable peaks at 2919 cm^{-1} and 2850 cm^{-1}, confirming the presence of C-H stretch in alkanes. Tarmat samples from west and south coasts of Qatar are

characterized with weakly condensed aromatic structures compared to those found in north and east coasts. Northern and western coasts showed a higher quantity of aliphatic composition (1.4509 and 1.1921) compared to the eastern and southern coasts (0.5498 and 0.1706). Microbes associated with tarmats are stated to have toxic degradation capabilities for the hydrocarbons. Hence, diversity of bacterial communities associated with tarmats along Qatar coastline have studied based on V3–V4 regions of 16S rRNA gene sequenced using Illumina Miseq Platform . Phylum Proteobacteria were found dominant in all the tarmat sample studied. Identified abundant genera include *Alkalimnicola, Alkanivorax, Marinobacter, Petrotoga, Defluvicoccus* and *KCM-B112. Marinobacter* and *Alkanivorax* are the major hydrocarbon degraders from the observed bacterial communities. Estimation of diversity indices have proved northern coasts are higher in bacterial diversity. This is the first metagenomic study of tarmat associated bacterial community in Qatar.

DEDICATION

With my genuine gratitude and warmest respect, I dedicate this thesis to my father who has always inspired me to pursue my studies.

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LIST OF ABBREVIATIONS

ASV – Amplicon Sequence Variants

ATR-FTIR - Attenuated Total Reflection Fourier Transform Infrared Spectroscopy

CFU – Colony Forming Unit

EEZ – Exclusive Economic Zone

GC-FID – Gas Chromatography – Flame Ionization Detector

GC-MS – Gas Chromatography-Mass Spectrometry

GDP – Gross Domestic Product

HMW – High Molecular Weight

HPTLC – High Performance Thin Layer Chromatography

LMW – Low Molecular Weight

MALDI-TOF MS – Matrix Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry

MECO – Middle East Crude Oil

OTU – Operational Taxonomic Units

PAHs – Polycyclic Aromatic Hydrocarbons

PCR – Polymerase Chain Reaction

SOM – Submerged Oil Mats

SRB – Surface Residual Balls.

TMSM – Tarball Mineral Salt Medium

TPH – Total Petroleum Hydrocarbon

UVF – Ultraviolet Fluorescence

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Oil pollution in recent decades, especially in the Arabian Gulf since the Gulf war oil spill of 1991, is considered as one of the most persistent threats. Industrialization and urbanization have intensified the oil and gas exploration, and subsequently the tanker traffic too, leading to oil pollution in the marine environment.

Crude oil is a mixture of both hydrocarbon and non-hydrocarbon compounds and has potential impacts on the ecosystem. Spilled crude oil is subject to different weathering processes in the ocean and eventually breaks into small pieces. Most of the heavier compounds that cannot be evaporated will be re-transported by winds, waves, and currents to form a densely weathered tarmats and tarballs after mixing with water to form a thick emulsion (Fingas and Fieldhouse, 2004; Goodman, 2003). These are weathered remnants of petroleum, floating in the ocean or deposited on the coast and derived from both natural and anthropogenic sources. Their concentration and composition vary and used to identify the source and origin. In addition to adverse economic effects, including recreational and tourism activities, the formation or accumulation of tarballs or tarmats in the marine environment is the ultimate fate of spilled oil and it is worth finding out its impacts in the marine environment. Oil spills occurring on the shore usually have more effects on various environmental matrices, including water and sediment.

Oil spill is one of the major sources for the distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in the marine environment. PAH has harmful biological effects, including toxicity, mutagenicity, and human health issues. To conduct an appropriate risk assessment and risk management practices, it is vital to know the effects on different environmental matrices of specific emission sources. PAH diagnostic ratios

are, therefore, considered as important parameters to classify the causes of pollution. To monitor the long-term impact of the spill, it is crucial to identify and characterize oil residues.

The Arabian Gulf oil discovery has enriched the economy of the Gulf countries but may lead to marine environmental problems if proper remedial measures are taken as and when needed. Qatar could be viewed as a model country for examining issues related to oil spill and biodegradation of oil components due to its harsh soil and climate conditions. Historically, various-scale oil spills existed in the area, both due to war disputes and accidental spills. The Kuwait oil spill in the early 1990s due to conflict events was one of the world's most massive oil spills. Invasive animal species, climate change, pollution, and habitat changes are the four main threats to global biodiversity listed in Qatar's convention on biological diversity. The oil discovery has led to socio-economic and environmental changes in the area. Crude gas and oil in Qatar represent the third-largest gas reserves in the world (Planning Council, 2007) and comprises of Qatar's 62% of GDP, the fastest growing economy in the Gulf coastal countries.

Photooxidation, chemical oxidation, volatilization, bioaccumulation and adsorption are the various processes that allow oil residues to be removed from the environment. Microbial biodegradation is indeed a more efficient and environmentally and sustainable friendly process for the removal of oil residues. Its metabolic plasticity enables a broad range of microbial communities to become capable consumers of complex substrates of hydrocarbons and, if used correctly, can result in efficient removal. In this work, we would like to investigate various types of microbial communities present in the tarmat samples collected from different locations of the Qatar coast.

1.2 Tarmats along the Qatar Coast

One of the phenomenon that affects the coastal environment of Qatar is deposition of oil residues, namely, tarballs and tarmats. Tar balls are oil fragments (not necessarily ball shape) that have solid/semi-solid consistency and are roughly spherical and >10 cm in diameter; <10 cm in diameter are termed as tar patties. During weathering process, when the emulsified oil interacts with suspended solids, it sinks to the sea bottom, and forms an immobile submerged oil mats (SOMs), called tarmats, while the moving/floating surface residual balls (SRBs) are called tarballs (Hayworth and Clement, 2011). Pollution events resulting from the rapid expansion of the oil industry has resulted in oil contamination in several Arabian Gulf beaches. Also, past conflicts that have damaged oil wells (e.g., Kuwait war) has discharged large volumes of crude oil into this marine environment. The source oil required for tarball/tarmat formation need not be due to major shipping incidents such as collision, grounding, fire and oil well blow out, it could be even due to continuous operations such as berthing, bunkering, other terminal operations, accidental discharges and natural seepages. One can easily locate the tarmat deposits along the Qatar coast when we have a walk on the coastline, especially on the west coast.

CHAPTER 2: LITERATURE REVIEW

2.1 Sources of Oil Pollution

The sources of spills in the sea surface are offshore oil exploration (Clark, 2002), oil tanker accidents (Baker and Dicks, 1982; Golob and Brus, 1984), oil-well blowouts (NAS, 1975; Wong et al., 1976; Atwood et al., 1987), accidental and deliberate release of bilge (Chandru et al., 2008) and ballast water from ships (Law, 1994 ; Ehrhardt and Blumer, 1972). The contribution of foreign vessels for oil pollution should be as well highly considered. For example, around 50,000 foreign ships ply the Malaysian waters making it highly trafficked and accident-prone (Chandru et al., 2008).

The Arabian Gulf region includes Oman, the United Arab Emirates, Qatar, Bahrain, Saudi Arabia and Kuwait, and most of them have extensive reserves of crude oil as the growth of their economy relies heavily on petroleum and gas exports. There are around 800 offshore oil and gas platforms and 25 major oil terminals with a busy shipping line for oil transport such that 60% of oil transportation through ships occurs annually, hence accidental oil spill is entirely unavoidable which results in heavy contamination of shores with trace metals, tarballs and oil residues. An annual spill of around 2 million barrels of oil happens due to a lack of operational facilities (Kirby and Law, 2008). Qatar has 25.7 billion barrels of oil reserves. Nearly all their oilfields are inland, with the only exception being the Dukhan region. Most condensates and NGLs in Qatar are extracted from the north area (from https://theoilandgasyear.com/market/qatar/). The major sources of oil pollution in the coastal environment of Qatar are industrial discharges, accidental spills, oil exploration activities and chronic discharges (Aboul Dahab and A1-Madfa, 1992).

Fowler (1985) carried out 18 months of significant work on coastal pollution in Arab Gulf coastal waters. His work documented the highest recorded tar loads on Bahrain

beaches (15-858 g/m^2) and the lowest tar loads on Abu-Dhabi beaches (4-232 g/m^2). Due to 1991Gulf War oil spill, 460 million gallons of oil spilled between August 1990 and February 1991. In addition, 500 million barrels of leaked or inflamed oil was added to the Gulf as aerosols, soot, toxic fuel and gases, even though a small amount of these pollutants had accumulated within the coastal environment (Fowler, 1993).

2.2 Formation of Tarmats and Types of Tar residues in the Marine Environment

2.2.1. Formation of tarmats

A wide range of physical, biological and chemical mechanisms such as evaporation, emulsification, dispersion, sedimentation, oxidation and biodegradation are responsible for the formation of tarmat / tarball. These weathering processes determine the fate of spilled oil in the marine environment. For instance, most hydrocarbons with lower molecular weight would evaporate, while gravity forces may deposit hydrocarbons with high molecular weight.

In some cases, oil compounds remain on the seabed, or can form water–in oil emulsion, through several environmental interactions. Such emulsions are the precursors for tar mats, and heavier oil with high viscosity form emulsions faster than lower weight oil residues (Payne, 1982). This emulsion would eventually disappear into smaller lumps or large oil mats, commonly called as chocolate mousse, and would be carried to various beaches and coastal areas via winds, waves and tides. Aggregation of small oil flakes to the tar residues increases their size over time through the agitation of wind and waves by adhering to each other (Payne, 1982). Usually found in small spherical forms or as hard mats, can be sticky and vary widely in color, shape, size, chemical composition and aroma. Tarballs accumulate along beaches or shores depending on various characteristics such as beach structure, type, quantity of spilled oil, currents, winds and waves. Tarballs would stay on the beach until physically or

biologically destroyed by drifting sand or sediment (Abu-Hilal and Khordagui, 1993). Romero et al. (1981) found that wind intensity and the amount of sand drifted has affected the buried stranded tar in Florida beaches. Tarballs are also quite dispersed with increasing temperatures and penetrate deep into the sand, which may ultimately transform the natural color of sand into blackish oil-slicked sand (Rekadwad and Khobragade, 2015).

2.2.2 Types of tar residues

In literature, tar residues are generally mentioned as Submerged Oil Mats (SOM) or Surface Residual Balls (SRB). Surface balls are typically known as tarballs with a diameter of less than 10 cm (OSAT-2, 2011; OSAT-3, 2013). Submerged oil mats or sediment oil mats generally are accumulated on the sea-bottom around 1-10 meter

(Michel et al., 2013) in size, and typically consist of a dense mixture of oil, sand and shell. Residual tarballs generally comprise a secondary weathering deposit of more massive oil deposits (Hayworth et al., press; Michel et al., 2013). Tar residues are also categorized into pelagic and benthic residues based on the ability to float at sea or reside at sea. While undergoing processes such as continuous weathering along with changes in ambient temperature, pelagic tarball might become benthic through the colonization of barnacles and isopods. (Iliffe and Knap, 1979). Even if being encrusted with sediments, inside may be soft and gooey, and high-temperature conditions can re-liquefy the residues (Hooper, 1981; Georges and Oostdam, 1983; NOAA, 2010). In the Arabian Gulf, tarballs are exposed to relatively high evaporation and photo-oxidation due to large variation in sea and air temperature, which induces formation of heavy petroleum tarballs. (Badawy et al., 1993).

2.3 Distribution and Occurrence of Tarmats in the Arabian Gulf

Due to the unique environmental conditions, extensive investigations such as surveying tar and marine pollution on beaches of Kuwait (AI-Harmi and Anderlini, 1979), Oman (Burns et al. 1982) and Saudi Arabia (Price et al.,1987; Stephen and Gunay 1989) have been carried out. High concentrations of dissolved petroleum hydrocarbons were observed from a survey conducted by El- Samra et al. (1986) along the northern coast of Qatar. Aboul Dahab and Al- Madfa (1993) indicated that possible sources of oil on the coast of Qatar are external to the west, but local to the east coast. Most of the earlier studies observed low concentration levels off southwest of Qatar, considering factors such as physical processes and biodegradation, whereas northwest of Qatar showed high concentration (Al-Lihaibi and Ghazi, 1997). A study by Burns et al. (1982) showed a north-south trend of decreasing tar balls along the Oman beaches. However, later Oostdam (1984) confirmed that tarballs observed larger than 25 cm in dia. along the Masira Island was among the highest size recorded worldwide. Tarballs were later found at about 77% of 53 coastal sites in the Gulf, with large quantities of tar at most locations (Price et al., 1989; Price, 1993). Al Madfa (1999) recorded average tar concentration between 723g/m and 620g/m along the northwestern and northern coasts of Qatar, respectively. After the Gulf war oil spill, Qatar coastline (especially, northwest coast) was severely affected by tarmat contamination, where the UNESCO world heritage site Al Zubarah is situated. The tarmat sampling survey in June 2015 found that both weathered oil from the Gulf War and fresh oil from recent oil spills contaminated the beaches in the Gulf (Al-Kaabi et al., 2017).

2.4 Chemical Composition of Tarmats

The composition of crude oil is highly variable; different forms are present in the carbon, ranging from C5 to C40 in related quantities of paraffinic, aromatic,

asphaltene compounds, significant differences in n-alkane distribution and relative isoprene ratios. These variations in the composition depend on the carbon sources produced by the oils and the geological environment and reservoirs from which these residues are migrated (Fingas and Wang, 2003). As a result, tarmats can consist of complex mixtures of hazardous chemicals, including saturated hydrocarbons, aromatic hydrocarbons, resins, asphalt, etc.

2.4.1 Polycyclic Aromatic Hydrocarbons (PAHs)

USEPA (1993) listed PAHs as hazardous organic compounds, and over 100 of the identified PAHs are considered priority pollutants. Sources of PAH in nature usually comprise of petrogenic and pyrogenic PAH. Pyrogenic PAHs are formed when organic compounds are subject to elevated temperatures of about 350°C to over 1200°C under reduced oxygen or no oxygen pressures by pyrolysis. These are usually found in higher concentrations in urban areas and near major PAH sources. PAHs produced during crude oil maturation are referred to as petrogenic. These petrogenic PAHs are common due to the widespread use of crude oil and crude oil products for shipping, transportation, and storage (Abdel - Shafy and Mansour, 2015). Usually, petrogenic PAHs are delivered directly to a water body from and combinewith sediments, whereas pyrogenic PAHs are released into the air first.

PAHs can be classified into Low Molecular Weight (LMW) and High Molecular Weight (HMW) based on their molecular weights and number of aromatic rings. Naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, and phenanthrene are some of the LMW PAHs with two or three aromatic rings, and fluoranthene, pyrene, benzo[a]pyrene, and perylene are some of the HMV PAHs with four or more aromatic rings. Low molecular weight PAHs are usually formed during low-temperature processes, while high molecular weight PAHs, which are less

alkylated, are emitted during high-temperature reactions (Mostert et al., 2010; Hwang et al., 2003). The hydrophobicity and electrochemical stability of a PAH molecule depend on their size and angularity (Zander, 1983; Harvey 1997), which are the two key factors that determine their environmental persistence. LMW hydrocarbon degradation performance will always be higher than that of HMW hydrocarbons as LMW undergoes a faster weathering phase.

Although the above-mentioned chemical components are characteristic of tarballs, their concentrations vary from one source to another. Tar residues also contain semi-volatile organic compounds such as hopanes and steranes, which are highly resistant to degradation; hence used as geological markers in addition to PAHs (Peters et al., 2005). Heterocyclic hydrocarbons of nitrogen and oxygen are another group of significant hydrocarbons found in oil residues. These are present at deficient concentrations compared to PAHs but can be oxygenated and regenerated in the environment with increased weathering because of exposure to light over a long period and has characteristics of high boiling fractions. Carbazole, quinoline, and pyridine were also found in individual and alkyl homologs in crude oils (Wang and Fingas, 2003).

2.4.2 Significance of diagnostic ratios

Concentration ratios of different pairs of PAHs are widely used in qualitative determination of PAH in air, soil and water. These are based on either parent PAHs or alkyl-substituted amounts for non-substituted molecules which apply to several environmental matrices. These are also used to distinguish between PAH pollution caused by petroleum products, gas combustion, and biomass or coal combustion, and the values will vary based on transition variations and environmental degradation. The mixture of molecules may have identical physicochemical properties because of the

resemblance of the molar mass (McVeety and Hites, 1988). Three-ring compounds are mainly used for source identification because of their presence in petroleum and many of its refined products at a suitable concentration. Identifying the source of oil is the first step to evaluate their causes and to establish effective pathways for remediation mechanisms. Implementation of pollution control regulations aimed at protecting public health and the environment also require effective methods of study.

2.5 Biodegradation of Hydrocarbons

2.5.1 Factors Influencing the Microbial Degradation of Hydrocarbons

Biodegradation affects various abiotic influences, including pH, redox potential temperature, moisture, oxygen, nutrients, soil salinity, and hydrocarbon mixture composition (Bamforth and Singleton, 2005). In soil, the degradation cycle will be more complicated as hydrocarbons are highly absorbed and thus become partly active in the soil. This would make hydrocarbons partially unavailable to microorganisms, which indicates further bioavailability as a critical element of biodegradation (Elazzazy et al., 2015).

Oxygen: Initial actions in the catabolism of hydrocarbons by bacteria and fungi in the aliphatic (Singer and Finnerty, 1984), cyclic (Perry, 1984) and aromatic (Cernigilia, 1984) require molecular oxidation of substrates. For this route of microbial oxidation of hydrocarbons in the environment, aerobic conditions are needed. To fully oxidize a hydrocarbon into carbon dioxide and water, three or four parts of dissolved oxygen are needed. Oxygen is available depending on the level of oxygen consumption by microbes, soil quality, porosity and the nature of the available substrate, which may result in oxygen depletion (Bossert and Bartha, 1984). Anaerobic oxidation of certain hydrocarbons also takes place, usually at small levels. This degradation involves different chemicals and is generally considered to be little ecological significance.

Nutrients: Though oil is rich in carbon sources, mineral nutrients needed to support microbial growth are lacking (Aeckersberg et al., 1991). Several studies showed a slow rate of biodegradation by an insufficient supply of these nutrients (Pruthi and Cameotra, 1997). The lack of nitrogen and phosphorus most likely reduces biodegradation, but the lack of iron or other trace minerals can sometimes be significant. For example, iron is more limited in clear, offshore waters than in sediment-rich waters.

Salinity: Shiaris (1989) recorded a generally positive association between salinity and phenanthrene and naphthalene mineralization levels in estuarine sediments. In an analysis of hypersaline salt evaporation ponds, Ward and Brock (1978) observed that the hydrocarbon metabolism levels dropped when salinity levels increased from 3.3% to 28.4%, resulting in an overall decrease in the microbial metabolism rates.

pH: Unlike most aquatic ecosystems, soil pH in alkaline deserts can be as high as 11.0 (Bossert and Bartha, 1984). Most heterotrophic bacteria and fungi favor a zero-like pH, with fungi more prone to acid (Atlas, 1988). Hence, the high pH of some soils will harm the degradation capacity of microbial species for hydrocarbon degradation.

2.5.2 PAH and Alkane Biodegradation by Bacterial Communities

A wide array of micro-organisms, including fungi and algae, is well adapted for PAHs and hydrocarbon biodegradation (Luo et al., 2014). Oil weathering processes affect biodegradation, which could even change the oil composition and diminish the bioavailability of these compounds. Other factors that could limit the biodegradation caused by weathering include an increase in hydrophobicity and the release of toxic chemicals. The main types of microbial degradation include complete mineralization, co-metabolic transformation, and non-specific oxidization and aerobic microorganism-mediated biodegradation of PAHs (Cerniglia, 1984). It is important to note that a

combination of hydrocarbons (PAHs) contaminates the atmosphere, so it is vital to identify micro-organisms which are very stable under a wide variety of environmental conditions. The limited area-to-volume ratio of tarballs can also obstruct the bioavailability (Atlas, 1981; Leahy and Colwell, 1990). The polycyclic aromatic compounds degrade at different rates concerning water solubility, volatility, biodegradability, and the degree of exposure.

The degradation of hydrocarbons by the metabolic pathway is mediated by genes that encrypt enzymes such as alkane hydroxylases, monooxygenases and P450 oxygenases (Das and Chandran, 2010). Some CueO and CotA bacterial laccases from *Escherichia coli* and *Bacillus subtilis* are capable of oxidizing PAHs, but rely on oxidation levels and copper dependence (Zeng et al., 2016). The isolation of 53 strains of PAH-degrading bacteria consisting of 14 strains of phenanthrene (Phe), 13 strains of Pyrene (Pyr), 13 strains of benzo[a]pyrene (Bap), and 13 strains of blended PAH (Phe + Pyr + Bap)-degrading bacteria was documented. The study showed that 91% of Phe and mixed PAH degrading consortia exhibited the highest capacity for degradation, relative to Pyr biodegradation by the Pyr-degrading consortium in 3 days and Bap by Bap-degrading consortium. Deepwater Horizon Oil spill analysis established uncultured *Marinobacter* in the halophilic soil consortium as a key phenanthrene degrader (Dastgheib et al. 2012). Consequently, the degradation of PAH will rely very much on both the bacterial community and the PAH compound. Although the main objective of bioremediation is the principle of hydrocarbon reduction, the microbial operation cannot still be entirely effective in reducing to a complete degree of mineralization due to specific considerations such as toxicity and nature of the molecules. Oualha et al. (2019) reported that when optimized conditions were introduced, the indigenous bacterium *Bacillus sonorensis* played a crucial role in

biodegradation. The study indicated the importance of nutrients in the bioremediation process; when ammonium nitrate was used as a source of nitrogen, removal of PAH after 160 days was 32.4%, while urea inhibited the process of oil degradation and raised the pH to 9.55.

2.5.3 Diverse Bacterial Communities in Tarmats

Microorganisms, especially bacteria, can turn organic molecules into substrates metabolized by other organisms as a carbon and energy source (Mittal and Singh, 2009; Johnsen et al., 2007). Some may use chemotaxis to facilitate access to hydrocarbons via chemoreceptors and pathways (Samanta et al., 2002). Biodegradation by both aerobic and anaerobic organisms can occur spontaneously through natural attenuation (Karamalidis et al., 2010). However, incomplete biodegradation may lead to more toxic pollutants.

The marine or beach-sand microbes are tied to tarballs/tarmats and may influence the biodegradation of hydrocarbon-rich tarballs/tarmats. As a substrate for the physical attachment of different bacteria, tarballs can be used during their hydrocarbon degradation to promote microbial growth. A different growth profile of microorganisms has been reported from tarballs inclusive consumers such as chromobacterium microorganism Tarballs (Itah and Essien, 2005) and pathogenic bacteria such as the Vibrio (Tao et al., 2011; Liu and Liu, 2013). Tao et al. (2011) reported a high number of *Vibrio vulnificus* from tarballs of Alabama and Mississippi beaches about ten times higher than in sand and a hundred times higher than those from seawater. *V. Vulnificus* is a human pathogen known to be abundant in the Gulf coastal environment and play a crucial role in the carbon cycle and are highly efficient in degrading PAHs (Thompson et al. 2004). Rekadwad and Khobragade (2015) suggested that microorganisms such as *Micrococcus, Rhodococcus,* and *Pseudomonas*-like species are modern biological

techniques to treat hydrocarbons and tarballs from the contaminated area. However, due to their limited integration into DNA identification and phylogenetic characterization, the taxonomy of tarball microbes needs improvement (Bacosa et al., 2016; Nkem et al., 2016). Table 1 represents a list of bacterial taxa reported from tarballs.

Table 1. Taxonomy of Bacteria Reported from Tarballs

S. No	Study area and sampling location	Taxon/clade	Reference
1	Alabama and Mississippi beaches	• *Vibrio vulnificus*	Tao et al., 2011
2	Bight of Bonny (Nigeria).	• *Chromobacterium violaceum,* • *Cladosporium resinae,* • *Bacillus submarinus,* • *Micrococcus,* • *Pseudomonas aeruginosa,* • *Candida marina* • *Saccharomyces estuary* • *Corynebacterium glutamicum,* • *Nocardia marina,* • *Cryptococcus albidus* • *Vibrio parhaemolyticus* • *Escherichia coli*	Itah and Essien,2005
3	Northern Gulf of Mexico	• *Proteobacteria*.	Liu and Liu, 2013
4	Rhu Sepuluh Beach, Malaysia	• *Acinetobacter baumannii* • *Cellulosimicrobium cellulans*	Nkem et al., 2016
5	Galveston and Mustang, USA	• *Alcanivorax* • *Psychrobacter* • *Pseudoalteromonas, Oceanospirillales,*	Bacosa et al., 2016
6		• *Proteobacteria,* • *Actinobacteria,* • *Firmicutes,* • *Bacteroidetes*	Shinde et al., 2018
7	Goa , India	• *Marinobacter,* • *Halomonas,* • *Pseudomonas* • *Petrobacter* • *Pseudoalteromonas,* • *Cobetia* • *Glacieola* • *Vibro,* • *Staphylococcus,* • *Klebsiella,* • *Rhodococcus* • *Acinetobacter*	Fernandes et al. 2019

2.5.4 Hydrocarbon Degrading Bacteria of Soils in Qatar

The complexity of biodegradation will be higher in arid areas such as Qatar due to weathering processes (Al Kaabi et al., 2017). Because of the unique harsh desert conditions in Qatar, the development of adaptation routes for selection and local domination would be difficult for microorganisms (Al Disi et al., 2013). These conditions, however, would allow microbes to adapt to a unique biodegradation pathway. Recent studies showed that bioremediations and biodegradation strategies should be based on native-specific bacterial activity, their adaptation, diversity such that appropriate stimulation may be needed (Attar et al., 2017; Al Disi et al., 2017; Al Kaabi et al., 2017). Many bacterial isolates gained attention in terms of their biodegradation capacity. Microorganisms such as *Burkholderia, Arthrobacter, Pseudomonas, Mycobacterium, Rhodococcus,* and *Sphingomonas* were involved in the alkyl aromatic degradation phase. One of the most proven hydrocarbon-degrading bacteria is *Pseudomonas aeuroginosa*. The variability and metabolic function of P.aeuroginosa samples from 7 contaminated oil sites in Qatar have been examined by Al Attar et al. (2017). The study indicated a high 90% rate of hydrocarbon removal for molecular compounds, which further shows the importance *of Pseudomonas auerogino*sa in weathered oil remediation. Al Disi et al. (2017) recorded a total of 39 isolates, in which *Pseudomonas* and *Citrobacter* isolates have efficient degrading capacity from short-chain n alkanes to longer chain n alkanes with alternating composition of nitrogen source and C/N ratios. Al Kaabi et al. (2018) isolated hydrocarbon-degrading bacteria Bacillus and Pseudomonas from the oil-contaminated soils of Qatar. The sites investigated are extreme in terms of temperature, weather, soil, UV radiation and salinity, which were the leading causes of limited bacterial diversity. Results showed that *Bacillus sonorensis* could degrade hydrocarbons of the low,

medium and high molecular weights upto 89%, 61% and 92%, respectively.

2.6 Laboratory Analyses of Tarmats

2.6.1 Chemical Analyses of Tarmats

A wide range of instrumental and non- instrumental techniques are widely used to analyze petroleum hydrocarbons, including gas chromatography-mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), infrared spectroscopy (IR), supercritical fluid chromatography (SFC), ultraviolet chromatography (UV), thin-layer chromatography (TLC). GC approaches are the most commonly employed.

In any oil spill study, the most critical task is to classify the oil source in different kinds of petroleum residues (Stout, 2016; Zakaria et al., 2000). Zakaria et al. (2001) employed a two-step procedure for the separation of aliphatic and aromatic fractions, consisting of a clean-up phase column accompanied by a fraction column and subsequent hydrocarbon injection into GC-FID and GC-MS. The solvent mixtures used for the extraction are selected based on the solubilization potential and optimum extraction of tarball/tarmat material. Dichloromethane and n-hexane are the most typical solvents used for extraction. Ultraviolet absorption and fluorescent spectroscopy are the most frequently employed detectors in the liquid chromatography technique, while flame-ionization, electron capture and mass spectrometers are used in the gas chromatographic method. The methods for quantification are highly complicated and expensive; however, it provides excellent flexibility and useful data most of the time (Lawal, 2017).

A few studies have been conducted to establish TPH concentration in sediments, bivalves and mollusks before the 1991 Gulf war oil spill. Using a fluorescence technique, Fowler (1985) calculated concentration of TPH in water off Bahrain and UAE. Grimalt et al. (1985) detected n-alkaline from sediments obtained on Shatt Al-

Arab on the northern part of the Arabian Gulf, using GC-MS method, and recorded dry sediment values between 3.3 and 18.8 μg/g. Conde et al (1996) noted that most of the tarball samples collected on the eastern Atlantic coast were determined to be Iranian crude oil, likely due to an Iranian tanker spill off the Moroccan coast in December 1989. Suneel et al. (2013a) performed detailed fingerprinting analyses on the tarballs along the Goa coast using biomarkers, carbon isotope analysis and diagnostic ratios, and confirmed the source of tarballs from tanker spills.

Other than PAH and alkanes, iron and paraffin content could be used to determine whether the tar originates from a ballast or petroleum storage tank as iron particles could be an implication to determine the source, as oil could be in contact with steel equipment (Payne 1982). GC's study of paraffin and iron content in tarballs revealed mainly the source of tarballs from tanker supplies (Butler and Harris., 1975; Wong et al., 1976).

The ATR-FTIR spectroscopy technique is highly sensitive, rapid and reliable method with minimum sample preparation to characterize the oil residues (Martin et al., 2010; Obinaju and Martin, 2013). The technique of FTIR spectroscopy gives reliable information on the chemical composition such as aliphatic and aromatic compounds, oxygen rates and polyaromatic compounds condensation (Boukir et al., 1988). FTIR spectroscopy also provides reliable information on the characteristics of different functional groups in the sample, and the extent of condensation related to polyaromatic chains. In general, this technique provides a structural characterization of the samples (Permanyer et al., 2002, 2005, 2007; Griffiths and de Haseth, 2007).

2.6.2 Biological Analyses of Tarmats

Culture-dependent studies neglect the current dynamics of natural ecosystems and future relationships between their components. Therefore, cultivation-independent

approaches have been widely used to classify relevant environmental groups of microbials in the polluting soils correlated with PAH depletion. The most commonly applied methods include population structural analysis focused on the amplification of the 16s rRNA-PCR gene, accompanied by fingerprinting (i.e., DGGE) (Vila et al., 2015).

A variety of microorganisms, which degrades different PAHs have been identified, and new pathways for PAH degradation have been developed. Still, further study is needed to explore microbe interactions within consortia that decompose PAHs, the regulatory mechanisms of various ring-structured PAHs and co-metabolization of PAHs. DNA–DNA hybridization was directly applied to identify and track critical species rescued from the environment (Sayler, G.S., 1985; Guo, C et al., 1997). Laurie and Jones (2000) established two distinct PAH Catabolic genotypes using quantitative competitive Polymerase Chain Reactions. This analysis revealed 16S rDNA sequence types that represented species closely linked to known bacteria degrading high molecular weight PAHs. The reaction of bacterial populations to oil exposure in nearshore waters along the northern Gulf Coast was investigated by targeting the 16S rRNA geneV6-V4 area and showed abundance of genus Alcanivorax, Alteromonas, Marinobacter, Winogradskyella and Zeaxanthinibacter (Newton et al., 2013). Throughout numerous studies, the next-generation sequencing of 16S rRNA genes has been used actively to test diverse bacterial diversity (Simon and Daniel, 2011; Wang et al., 2016). By producing high-performance data, this approach may provide current, accurate and reliable information on the taxonomic identification of microbes (Huber et al., 2007; Chen et al., 2013). Oualha et al. (2017) performed a biopile system design along with GC-MS, FTIR and CFU to address and classify the shortcomings of weathered oil hydrocarbon bioremediation using an adapted Bacillus sorensis strain.

Evidence suggested that the indigenous bacterium played a crucial role in the process of biodegradation.

2.7 Impacts of Tarmat Pollution on the Ecosystem

Tar pollution affects the environment and thus has a harmful effect on provisions on ecosystems supporting, cultural and regulatory services. Oil and tarball reserves are slowly exhausted in the seawater and sand with active chemo-heterotrophic microorganisms. Nevertheless, some endangered marine species and amphibians could be negatively affected. Beach ecosystems are indeed a natural link between water and soil, which, through anthropogenic intervention, can disrupt ecosystem stability and affect microbial communities. Beach habitats are also closely linked to the needs of human beings. Beaches can have profound implications for the local and regional economies, serving as the central physical location for human-marine interaction (USEPA, 2004).

2.7.1 Ecological Impacts

The marine habitats and species had different responses to the oil spills/tarballs/tarmats. Die-offs are found among some organisms, and, based on prevailing conditions, others like algae and mangrove are usually regenerated rapidly (US Fish and Wildlife Service, 2010). Since the circulation in the Gulf is not so strong, oil spill if it happens, it is disastrous for the Gulf environment. Even after 15 years, the Gulf had not recovered fully from the catastrophic Gulf War oil spill (Barth, 2007). Added to PHC content, high saline and high temperature of water in the Gulf are severe stressors for species, as organisms need to withstand such harsh conditions through osmoregulatory and thermal adjustment mechanisms for their existence.

There have been very few studies evaluating the ecological effects of tar residues. These include the physical coating of oil on birds contributing to smothering

or hypothermia, inhalation and absorption of toxic compounds due to high-volatility oil breakdown in water that can even trigger immune system (Peterson et al., 2003). Accidental consumption of pelagic tar can occur when marine turtles feed on the shore, but either adult or hatchling sea turtles feed onshore so that the risk of harm from beached tarballs/tarmats is minimal (OSAT-2, 2011). Starfish are another species that ingest the tar residues unintentionally. These organisms are found in high numbers in places where benthic tar is abundant, which could be for feeding the tar for their nutritional benefits as through selective feeding (Pequegnat and Jeffrey, 1979). A study comparing unpolluted beaches to dirty beaches showed a decline in mollusk abundance and diversity. Here, tar has a negative effect on the soil substratum that forms their habitat (Nagelkerken and Debrot, 1995).

Nevertheless, after the 1991 Gulf War spill, Poonian (2012) recorded no significant impacts on the seagrass and corals ecosystem probably due to the deposition of oil on the surface instead of undergoing extensive mixing by wind or wave action. Oil slicks and tar on the water surface damage aquatic vegetation by blocking sunlight and preventing adequate air circulation. Hardened tar can enter the subsurface that affects many animals and benthic environments.

Some in situ and lab experiments have shown that total toxicity will be reduced as the weathering occurs. The research investigated the impacts of DWH oil spillage on several wildlife habitats found that the majority of PAHs are depleted in oil (OSAT-2, 2011). A high-watered tarball on the sand cannot be ecologically dangerous as a freshly uncovered tarmat fragment.

2.7.2 Human Impacts

It is usually not possible for people to avoid exposure to sources of PAH. Inhalation and skin contact in industrial and non-occupational conditions are the major

routes of exposure (Sokhi et al., 2008). PAH treatment is known to cause eye irritation, diarrhea, nausea etc. The primary skin irritants and sensitizers are anthracene, naphthalene and benzopyrene (Unwin, et al. 2006). Long-term effects on health include breast, liver, blood and gastrointestinal cancer, damage to the kidneys and organs, cataracts, cardiopulmonary mortality, etc. Nonetheless, there are enough findings to show exposure of carcinogenic properties of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, and benzo(k)fluoranthene (Mastrangela et al., 1997; Liuet et al., 2001; Sramet et al., 1999). Pyrene is a toxic recalcitrant that is mutagenic and more harmful than its parent compound (Singh, 2006). When the head, liver, bladder and lymphatic network are inserted into the tissue, the studied tumor tends to be an anthracene (Das et al., 2008). Naphthalene is also an inhibitor of mitochondrial respiration; it covalently binds to molecules in hepatic, kidney and lung tissue, increasing its toxicity (Falahatpisheh et al., 2001). Phenanthrene is considered to be a photosensitizer for a human skin and a mild allergen (Mastrangela et al. 1996).

As mentioned earlier, tar mats can also serve as a reservoir for pathogenic bacteria, such as V. Vulnificus. It suggests a severe health risk, as direct contact with such tarballs/tarmats can lead to serious wound infections, which could lead to significant health conditions, such as secondary septicemia. This pathogen is also one of the leading causes of seafood-borne fatalities nationwide in the United States (Mead et al., 1999). Environmentally persistent-free radicals which are produced by the partial oxidation of ferrous embedded in sediment particles have been detected in the tarballs collected from the Gulf of Mexico, which are potentially very dangerous when accidentally ingested or inhaled (Kiruri et al., 2013).

2.7.3 Economic Impacts

Besides these, tarball could have adverse effects on the tourism sector. For example, the deposition of tarball on the Goa coast was of great public concern, as coastal tourism operations were severely affected (Suneel et al. 2013b). Similarly, oil pollution/tarmat along the Zubarah coastline is of great concern because of tourism and visual perception because Al Zubarah is on the UNESCO World Heritage List and is of great importance for the history of Qatar (UNESCO).

2.8 Significance and Scope of the study w.r.t the EEZ of Qatar

Several studies have been carried out to understand the bacterial hydrocarbon-degrading community in the Gulf region, but those from tarmats or tarballs are poorly understood and studied. No research has been carried out so far to understand the genomic patterns of bacterial, fungal and archaeal communities associated with tarmats in the Middle East.

Research conducted particularly in Qatar, which is related to oil spills or oil pollutants, involves identifying sources of beached oil (Al Kaabi et al., 2017) and characterizing the origin of biogenic and anthropogenic hydrocarbons in sediments in the northwestern region of Qatar (Rushdi et al., 2017). There are numerous studies, which focused on isolating microbial species and identifying them from various oil-polluted sites in Qatar. The effective biodegradation rate occurs at higher temperatures, so the warmer climate can be considered as an essential factor in determining degradation in the Gulf areas. The MALDI-TOF mass spectrometry was used to identify bacteria that degrade hydrocarbons from oil-contaminated soils in Qatar (Al Kaabi et al., 2018). Al Disi et al. (2017) evaluated the specific effects of severe weather and oil on the diversity, adaptation, and activity of hydrocarbon-degrading bacteria in bioremediation strategies for harsh oil polluted soils and has demonstrated clearly that

hydrocarbon-degrading bacteria in Qatar are abundant and diversified. However, until now no experiments or work has been carried out on microbial composition and propagation in tarmats.

The primary source of tarball pollution in Malacca Straits was from Middle East wastewater oil tankers (Zakaria et al., 2001). Preliminary quantitative investigations conducted on the shore of the Gulf have recorded about 1-10 kg/m of tar levels (Stephen and Gunay, 1989). Further, the fact that tar levels in the Gulf area were almost 100 times higher than that reported for other regions of the world is fascinating and evident (Price 1993). Large quantities of tar deposits have been recorded in the north and north-western parts of Qatar, including records of large amounts of Al-Zubarah tar deposits (Al-Kaabi et al., 2017). By understanding the ecosystem and human health impacts by tarmat contamination, new remediation techniques could be applied. In general, this work would, therefore, investigate the genomic variations in the bacterial populations of tarmats using a metagenomic method. Besides, molecular analytical instruments such as GC-MS has also been used for quantification of alkanes and hopanes, in addition to PAH quantification. The semi-quantification of Total Petroleum Hydrocarbons (TPH) in tarmats and their relative degradation rate have also been measured using FTIR spectroscopy. The methodology used in this research helps us to understand the type of microorganism capable of degrading PAHs and whether variations in tarmat sources affect the profile of the microbial diversity or not. An integrated approach to understand the composition of hydrocarbons and the bacterial community is vital, as the fingerprinting and understanding of the chemical structure of tarmat alone are not enough to trace sources of tarballs/tarmats and determine their fate.

2.9 Research Objectives

Since the last few decades, oil contamination has always been a major environmental issue; various studies have been carried out on oil spills to identify their sources through different physical, chemical and biological techniques. Tarmats are found heavily along northern regions of Qatar, but less research has been conducted to identify their sources, its formation and composition, remediation techniques or know their impact on ecosystems. The primary objectives of this study are -

- to collect the tarball/tarmat samples along the Qatar coast, where there is the presence of oil – rig installation
- to conduct *in situ* physicochemical analyses of sea water and the environment, such as pH, salinity and water temperature
- to determine and understand the genomic patterns of bacterial communities in the tarmats using a metagenomic approach
- to quantify the PAHs, alkanes, and hopanes using Gas Chromatography-Mass Spectrometry.
- to study the relative weathering pattern among the collected tar mat samples using ATR-FTIR spectroscopy.

CHAPTER 3: STUDY AREA AND MATERIALS & METHODS

3.1 Brief Description of the Study Area

3.1.1 Arabian Gulf

The Arabian Gulf (Fig. 1a) is located in the western arm of the Arabian Sea; it has a length of ≈ 1000 km and an average depth of 36 m; evaporation loss is higher than precipitation; water is highly warmer during summer; high saline waters of 36.5-37.0 enters the Strait of Hormuz on the surface and high saline water (>40) flows out through the bottom (Hunter, 1982). Because of large seasonal temperature difference (between summer and winter), there is seasonal density gradient currents, largely responsible for the transport of materials and suspended particles along with tide and wind driven currents. With high temperatures and faster evaporation rate, this region is remarkably a salty sea with a few rivers such as Shatt Al-Arab contributing freshwater to the Gulf. The salinity variations have also resulted in calcium sulfate and sodium chloride accumulation, creating vast coastal salt flats (sabkhas). In the basin, large amount of sediments, mainly calcareous and marls with evaporites and organic matter are deposited, which eventually produced the region's vast oil deposits. (https://www.britannica.com/place/Persian-Gulf). The region is home to a wide range of marine organisms including coral reefs, mangroves, and seagrasses offering different ecosystem services, including protection for marine species such as green turtles and dugongs.

3.1.2 Qatar coastal region

The state of Qatar is a small peninsula (11,000 km^2 area) which is generally flat and rocky and has a coastline of more than 700 km from the Salawa Bay at the border of Saudi Arabia to the border of UAE. Soils in Qatar are generally characterized by presence of small degree of organic material and more calcareous, making them

agriculturally less productive. Qatar has an annual rainfall of 81 mm. A hot and humid climate is observed from June to September with temperature as high as 50ºC and cooler temperature in winter even drop to below 10ºC. Higher evaporation rates and less water exchange with open oceans leads to more saline and hot conditions, hence pollutants in the Gulf region take more time to dissipate. Qatar is surrounded by relatively shallow water with an average depth ≈ 35 m. The average sea surface temperature of the coastal waters varies between 14°C in winter and 42°C in summer.

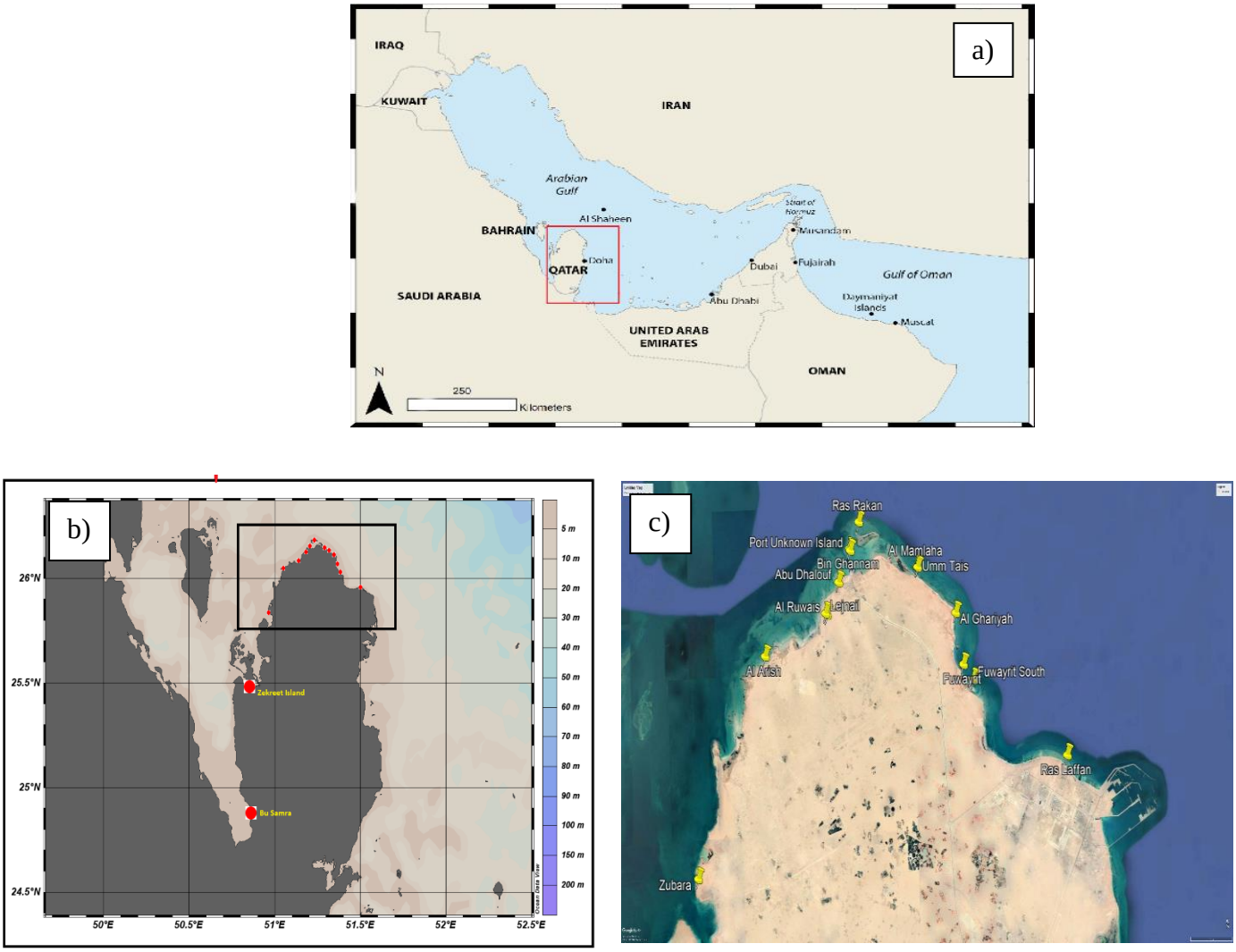

Figure 1. a) Study area in the Arabian Gulf, b) and c) Tarmat sampling locations along the Qatar coast

3.2 Sample collection

In order to carry out the present work, tarmat samples were collected from a few specific locations, more or less at every 10 km distance during the period May 2018 and September – November, 2019 using a stainless steel spatula, placed in labeled aluminum foil bags and stored in the laboratory refrigerator (–20°C). The sampling locations are shown in Fig 1.The coordinates of sampling locations are given in Table2

Table 2. Tarmat Sampling Sites along the Qatar Coast

S.No	Sample location	Latitude	Longitude	Date of sampling
1	Ras Laffan	25°56'42.00"N	51°31'19.70"E	30-May-18
2	Port Unknown Island (Near Ruwais Port)	26° 9'18.00"N	51°12'0.00"E	16-Sep-19
3	Umm Tais	26° 8'9.12"N	51°18'6.78"E	16-Sep-19
4	Fuwayrit	26° 2'18.60"N	51°22'4.50"E	16-Sep-19
5	Fuwayrit South	26° 1'23.60"N	51°22'57.30"E	16-Sep-19
6	Zubara	25°50'18.72"N	50°57'32.88"E	19-Sep-19
7	Ras Rakan (R1)	26°10'57.18"N	51°12'49.80"E	24-Sep-19
8	Bu Samra	24°50'49.00"N	50°51'43.00"E	30-Sep-19
9	Zekreet Island (West Island)	25°29'9.53"N	50°50'47.69"E	8-Oct-19
10	Al Mamlaha	26° 8'9.00"N	51°18'3.00"E	10-Oct-19
11	Al Ghariyah	26° 5'22.00"N	51°21'29.00"E	10-Oct-19
12	Al Arish	26° 3'16.00"N	51° 4'3.00"E	10-Oct-19
13	Lejnail	26° 5'39.00"N	51° 9'39.00"E	10-Oct-19
14	Abu Dhalouf	26° 7'27.00"N	51°10'53.00"E	10-Oct-19
15	Al Ruwais	26° 5'34.00"N	51° 9'47.00"E	17-Nov-19
16	Bin Ghannam	26° 9'23.00"N	51°11'55.50"E	17-Nov-19

3.3 Materials and Chemicals used

The organic solvents used in this study were of analytical grade or higher. Solvents dichloromethane and n-hexane were obtained from Sigma – Aldrich and Honeywell Riedel – de - Haen™ respectively. The tar residues with the solvent were mixed using Branson 200 Ultrasonic Cleaner. The extraction for hydrocarbon analysis was carried out using the Resprep™ Massachusetts EPH SPE 20ml/5g cartridge. Only glassware's were used for the analysis and all glassware were washed with detergents and distilled water and proper rinsing successively with methanol, DCM and hexane was as well carried out. A standard mixture consisting of Naphthalene,

Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene. Benz (a) anthracene, Chrysene, Benzo (b)fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Indeno (1,2,3 -cd)pyrene, Dibenz (a,h)anthracene, Benzo (g,h,i)perylene was used. The materials used for metagenomic analysis include QIAGEN Power Soil DNA Isolation Kit, QIAGEN DNeasy PowerClean Pro Cleanup Kit, Vortex, ThermoMixer Comfort, pipette, Nanodrop Spectrophotometer, Agarose and cyan/orange gel loading dye.

3.4 Experimental methods for metagenomics analysis of TM associated bacteria

3.4.1 DNA Extraction, Cleanup, Quantification and Gel Electrophoresis

A total of 16 locations were chosen and extraction was done in duplicates for 0.5 g tar mat samples. The extraction of DNA was carried out with 0.5 g of tar mat samples using QIAGEN Power Soil DNA Isolation Kit. Extraction was conducted according to the manufacturer's instruction. The extracted DNA were stored at -20℃. The DNA extraction for a few selected samples was carried out twice at different conditions. In order to check the presence of the DNA in the sample, a gel electrophoresis was carriedout using 1% casting gel stained with ethidium bromide and is viewed using a UV transilluminator. Gel ectrophoresis is done using Mupid Exu submarine electrophoresis system by casting 1% gel for 100v at 30 mins. 2µl TrackITTMof cyan/orange loading dye is mixed with 5µl of DNA sample. TrackItTM 50bp DNA ladder is used for the electrophoresis. DNA quantification for assessing the concentration was also carried out using Nanodrop Spectrophotometer. The samples that showed less integrity has been subjected to a cleanup procedure using QIAGEN DNeasy PowerClean Pro Cleanup Kit. Cleanup was conducted according to manufacturer’s instruction. Extraction, Cleanup and Quantification of the tarmat sample were done at Biomedical Research Centre, Qatar University.

3.4.2 Metagenomic Analysis

The extracted purified DNA samples shipped to Macrogen, Inc(South Korea) for metagenomic analysis. The metagenomics analysis using Illumina NGS workflow includes 4 basic steps:

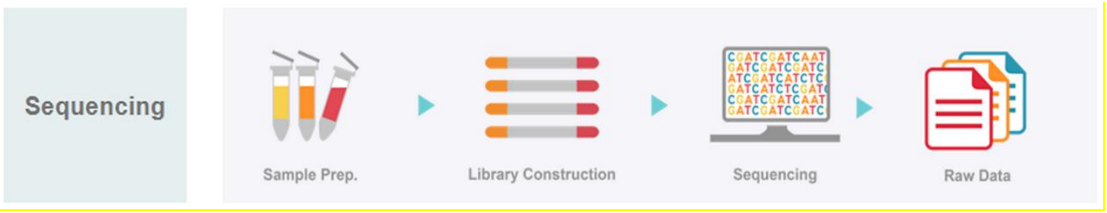

Figure 2. Experiment overview for library construction

Sample preparation: After performing quality control (QC) for the extracted DNA sample, qualified samples are proceeded for library construction.

Library construction: The sequencing library were prepared by random fragmentation of the DNA or cDNA sample, followed by 5' and 3' adapter ligation. Alternatively, "tag-mentation" combines the fragmentation and ligation reactions into a single step that greatly increases the efficiency of the library preparation process. Adapter-ligated fragments are then PCR amplified and gel purified. For the polymerase Chain Reaction, the V3 - V4 hypervariable region of 16s rRNA gene were amplified using the forward (341F) and reverse (805R) primers (v341 F: CCTACGGGNGGCWGCAG and 805 R: GACTACHVGGGTATCTAATCC) with the GC clamp set. Around a 25µl of PCR mixtures were prepared by using 2.5µl of DNA template, 5 ul of each primer and 12.5µl of KAPA HiFi HotStart ReadyMix. A DNA thermal cycler was used to carry out the PCR reaction. Initial denaturation temperature was set around 95 ° C for three minutes

which is followed by around twenty five cycles at 95 degree Celsius for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds. The final extension temperature is set around 72°C for five minutes. Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2 (Illumina Inc.) was used to prepare the amplicon library according to the 16S Metagenomic Sequencing Library Preparation Part # 15044223 Rev. B. Amplicons were ligated with Illumina sequencing adapters using the Nextera XT Index Kit and are purified by AMPureXP beads as per the standard Illumina protocol. The size of the enriched PCR fragments were verified by using Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip.

Sequencing: For cluster generation, the library is loaded into a flow cell where fragments are captured on a lawn of surface-bound oligos complementary to the library adapters. Each fragment is then amplified into distinct, clonal clusters through bridge amplification. When cluster generation is complete, the templates were ready for sequencing. Illumina SBS technology utilizes a proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands. As all 4 reversible, terminator-bound dNTP are present during each sequencing cycle, natural composition minimizes incorporation bias and greatly reduces raw error rates compared to other technologies. The result is highly accurate base-by-base sequencing that virtually eliminates sequence-context-specific errors, even within repetitive sequence regions and homo-polymers.

Raw data: Sequencing data is converted into raw data for the analysis.

3.4.3 Bioinformatics Analysis

Bioinformatic analyses, taxonomic assignment and other related statistical analyses were performed in R version 3.6.3 (R Core Team 2019). Sequence variants (SVs) were determined using the R package DADA2 version 1.6 (Callahan *et al*., 2016)

as follows: Demultiplexed and adapter-trimmed sequences were quality filtered using the filterAndTrim command and following setting: truncLen = c(290,210), maxEE = 2, trimLeft = 15, truncQ = 2. Learned error rates and sequence variants (SVs) inferred for samples from each run independently using the *learnErrors* command with a subset of 1 million reads and the *dada* command on both forward and reverse reads. Both read ends were merged using the *mergePairs* command with default parameters. Sequences 50 bp smaller than the expected amplicon size of 445 bp were removed and chimeras detected using the *removeBimeraDenovo* command with *consensus method*. Taxonomy was assigned with the *assignTaxonomy* command against a training set of the SILVA database version 132 (Henderson *et al*., 2019). In order to infer relatedness of SVs, they were clustered into Amplicon sequence variants (ASVs) / Operational Taxonomic Units (OTUs) at 99% identity threshold using the *cluster_smallmem* command of the UCLUST algorithm (Edgar 2010), sorted by read abundance. The Amplicon sequence variants (ASVs) were imported into the *R* package *phyloseq* (McMurdie and Holmes, 2013). Singleton SVs and SVs assigned as Chloroplasts were removed from the sequence table. Plots were created using R package *ggplot2* (Wickham, 2009). Dissimilarity matrices were calculated using the R package *vegan* (Oksanen *et al*., 2017) with method 'bray'. Hierarchical cluster plots were created using *hclust* of the R package *stats* with method "average".

3.5 Extraction and fractionation of PAHs

Approximately 0.1g of tar sample from each location was dissolved with 5ml of DCM in a test-tube. The well dissolved solution was transferred to another test-tube to carry out in duplicates. These samples were evaporated till dryness in the fume hood. The extraction cartridge was charged with 10 ml of hexane and eluent was discarded. Briefly, saturated fraction (F1) and aromatic fraction (F2) were eluted with 14 ml of

hexane and 15 ml of Hexane:DCM (50:50), respectively. Then, the F1 and F2 fractions were concentrated under a gentle stream of nitrogen and was adjusted to final sample volume of 1 ml in 2 ml glass vials.

3.6 Estimation of TPH using GC-FID

F1 fraction solutions were injected into an Agilent 7890B Gas Chromatograph coupled with flame ionization detector (GC-FID) to identify total petroleum hydrocarbons (TPH). A 30 m x 0.25 mm id (0.25µm film thickness) fused silica capillary column was used. The carrier gas used was helium (0.83 mL/min). The initial GC oven temperature of 40°C with 2 min hold was ramped to 330°C for 10 min at 10°C/min resulting in a total runtime of 41 min.

3.7 Estimation of PAHs using GC-MS

PAH analysis was performed on an Agilent 7890 Gas Chromatograph coupled with mass spectrometer (GC-MS). A 30 m x 0.25 mm id (0.25µm film thickness) fused silica capillary column was used. The carrier gas used was helium (0.83 mL/min). The initial GC oven temperature of 50°C with 0.5 min hold was ramped to 250°C at 25^0C/min and then ramped to 290^0C for 3.5 min at 5^0C/min, resulting in a total run time of 20min and the MS was operated in the electron impact mode at 70eV ion source energy. 16 PAHs containing Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene. Benz (a) anthracene, Chrysene, Benzo (b)fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Indeno (1,2,3 - cd)pyrene, Dibenz (a,h)anthracene, Benzo (g,h,i)perylene were analyzed.

3.8 ATR-FTIR analysis

Tarmat samples (TMs) were analyzed using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy (Thermo Scientific Nicolet iS10 spectrometer with Smart iTR Diamond crystal plate). TMs were sliced in half using a

solvent-rinsed razor blade and a small internal section (~1 cm dia.) was removed and placed on the ATR crystal for analysis. Absorbance spectra were recorded in the mid-infrared region (4000 – 600 cm^{-1}) using 32 scans at $2cm^{-1}$ resolution. A background atmospheric spectrum was subtracted from all sample spectra. Peaks were integrated using Omnic software.

Different indices that represent the structural and functional features of TMs were calculated on the basis of peak areas. Peak areas were measured from valley to valley (Permanyer et al., 2002). The following indices were calculated to compare the structural and chemical composition of TMs using peak areas (Asemani and Rabbani, 2015):

Aliphatic index: $(A_{1460} + A_{1376}) / (A_{2953} + A_{2923} + A_{2862} + A_{1700} + A_{1600} + A_{1460} + A_{1376} + A_{1030} + A_{864} + A_{814} + A_{743} + A_{724})$, which represents all aliphatic compounds present in a sample.

Aromatic index: $A_{1600}/(A_{814}+A_{743}+A_{724})$, which represents all aromatic compounds present in a sample.

Long chain index: $A_{724}/(A_{1460}+A_{1376})$, which represents straight chain alkanes with 4 or more carbon atoms in a sample.

Substitution 1 index: $A_{864}/(A_{864}+A_{1376})$, which represents benzene structures that share one H, which attach to C atoms of the benzene ring, with other structures. Polyaromatic compounds with low condensation have a Substitution 1 index greater than their Substitution 2 index.

Substitution 2 index: $A_{814}/(A_{864}+A_{814}+A_{743})$, which represents benzene structures that share three H, which attach to C atoms of the benzene ring, with other structures. Polyaromatic compounds with high condensation have Substitution 2 index greater than their Substitution 1 index.

In the above indices, ‘A’ refers to the peak area in the absorption spectrum and the subscript number represents the wavenumber.

CHAPTER 4: RESULTS

4.1. Distribution and physical characteristics of Tarmats along the Qatar coast

In this study, tarmat samples were collected from 16 beaches along the Qatar coast with major stations in the northern coastline. The study area includes some of the major populated coastal places such as Abu Dhalouf, Fuwairit, Ras Laffan, Al Ruwais, Zekreet and Zubarah. Tar residues were found as balls mixed with local marine litter and moderate quantities of seaweed. Northwestern coasts from Zubarah to Al Arish had huge and significant deposits of tarmats. Flat rock with thin sand layers and sabkhas formed most of the shores in this region. Most of the areas were heavily polluted with oil residues. The tarmats in the Qatar coast were spread over a large area and contained highly weathered, hard, asphalt-looking substances (Fig. 3).

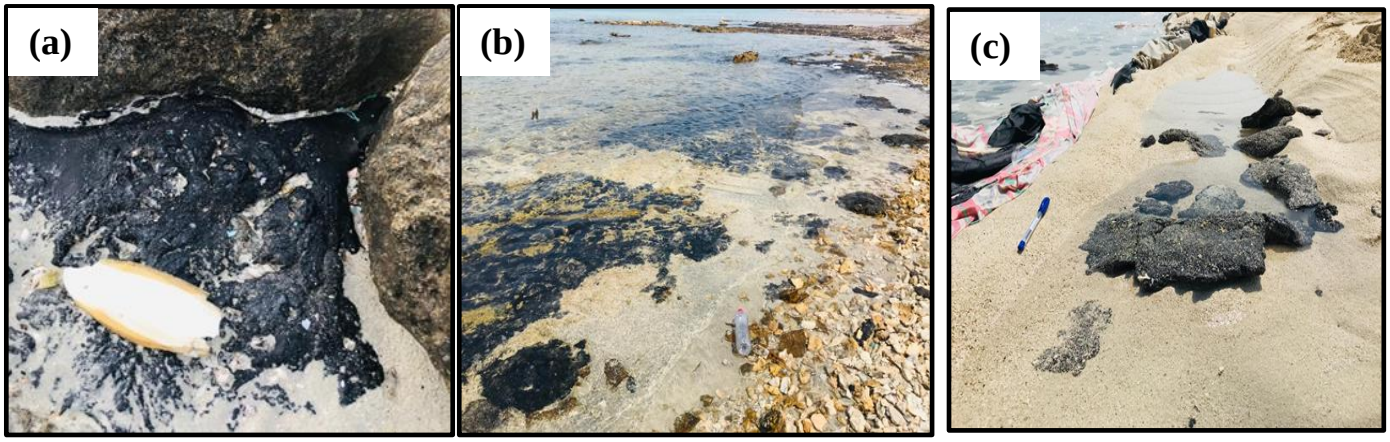

Figure 3. Tarmats observed during field sampling along the coast: (a) Abu Samra, (b) Al Zubara and (c) Umm Tais.

Han and Clement (2018) developed a field test protocol to classify Deep Water Horizon oil residues based on physical properties such as colour, odor, thickness, sand

content percentage, solubility level, etc. Similar protocol was applied in this study also to characterize and classify tarmat samples. Tarmat samples along the coast showed similar physical appearance with dark black or brownish color and low to high amount of sand, depending on locations (Fig 4.2). Most of the samples completely dissolved in DCM solvent, displaying less viscosity. Samples from Al Arish and Fuwairit have rough surface, whereas samples from Umm Tais and Zubara were stickier, smooth and gooey inside. Tarmat samples from Fuwairit, Al Arish, Ras Rakan, Al Ghariyah, Lejnail were less fragile and hard to break. On the other hand, samples from Ras Laffan, Zekreet, Bin Ghamnam and Abu Samra were highly fragile and easily broken. Most samples showed high to moderate petroleum odour (Fig 4).

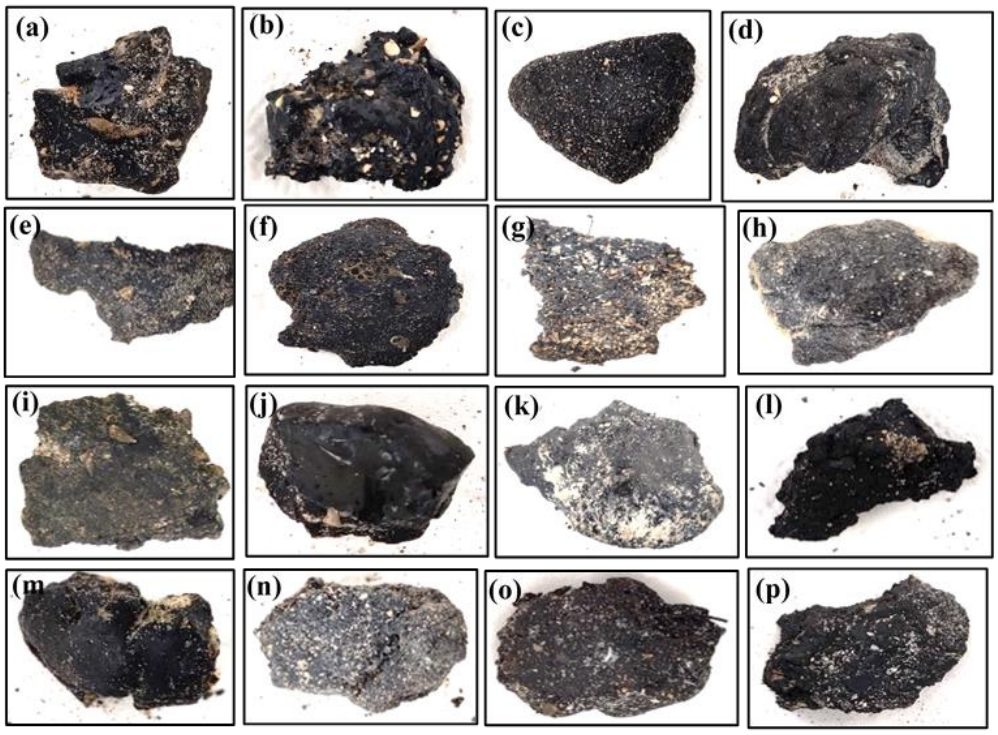

Figure 4. Tarmat samples collected from north (a-i), west (j-l), south (m) and east (n-p) coasts of Qatar

4.2 Concentration of TPH and PAHs in Tarmats

TPH concentrations in the study are illustrated in Fig 5. The locations included for the TPH analysis are Ras Laffan, Ras Rakan, Al Arish, Abu Dhalouf and Abu Samra.

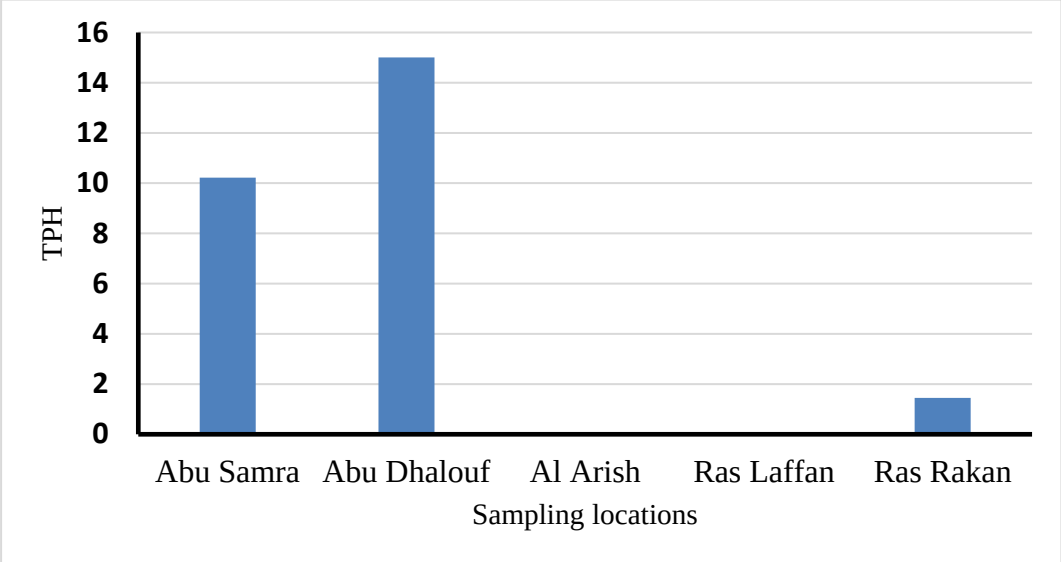

Figure 5. TPH levels (ppb) in tarmat samples along the Qatar coast.

Sixteen PAHs containing Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene. Benz (a) anthracene, Chrysene, Benzo (b)fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Indeno (1,2,3 -cd)pyrene, Dibenz (a,h)anthracene, Benzo (g,h,i)perylene were analyzed for tarmat samples. Fig 4.4 showed the concentration of 16 PAHs detected in ppb levels for tarmat samples determined through Gas Chromatography-Mass Spectrometer. Ras Rakan sample showed relatively higher levels of PAH and Abu Samra had the lowest levels of PAH (Fig 6).

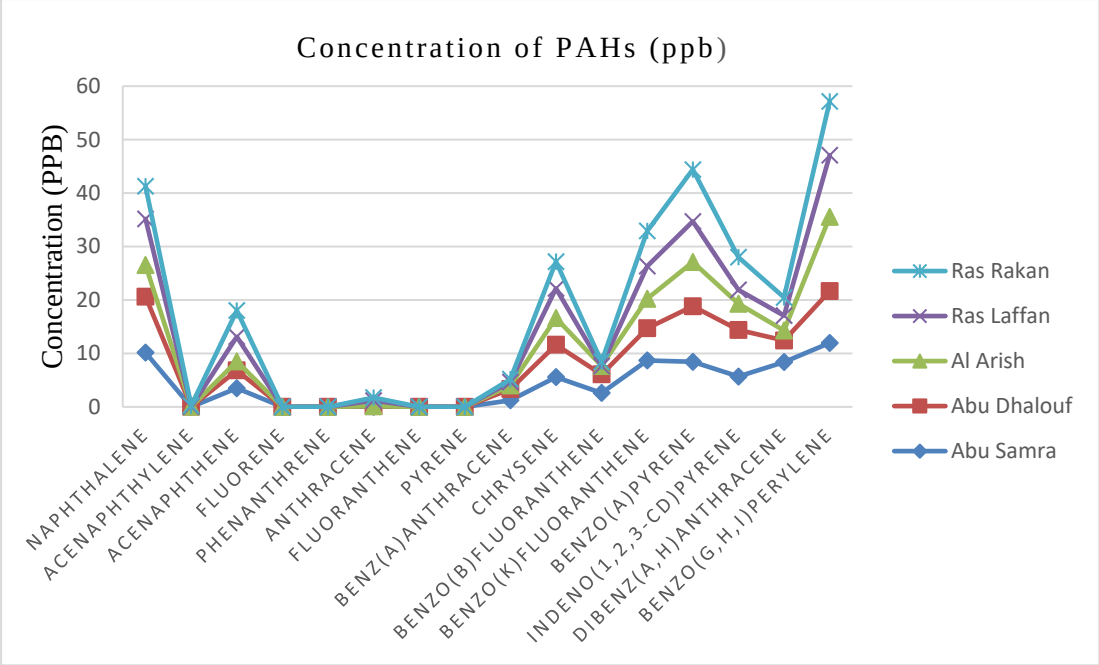

Figure 6. Concentration of 16 PAHs along the Qatar Coast

Table 3 provides the average concentration of 16 PAHs in tarmat samples from 5 locations. Benzo perylene showed the highest level of concentration (11.43 ppb) among all the PAHs, and this high value is observed for Abu Samra samples. Fluorene, Phenanthrene, Fluoranthene and Pyrene are completely degraded in all the samples. The results also indicate that tarmat samples from the location could have undergone an extensive biological and chemical degradation. Acenaphytlene and Anthracene comparatively showed lower concentration levels.

Table 3. Average concentration of 16 PAHs in Tarmat Samples from the Qatar Coast

Concentration of 16 PAHs (ppb)					
Identified 16 PAH compounds	Abu Samra	Abu Dhalouf	Al Arish	Ras Laffan	Ras Rakan
Naphthalene	10.160	10.465	5.907	8.630	6.110
Acenaphthylene	0.000	0.040	0.000	0.000	0.187
Acenaphthene	3.493	3.340	1.693	4.590	4.917
Fluorene	0.000	0.000	0.000	0.000	0.000
Phenanthrene	0.000	0.000	0.000	0.000	0.000
Anthracene	0.000	0.135	0.000	1.050	0.560
Fluoranthene	0.000	0.000	0.000	0.000	0.000
Pyrene	0.000	0.000	0.000	0.000	0.000
Benz(a)anthracene	1.230	2.105	0.690	0.637	0.523
Chrysene	5.583	6.035	5.010	5.533	5.023
Benzo(b)fluoranthene	2.610	3.480	1.563	0.000	0.777
Benzo(k)fluoranthene	8.687	6.025	5.513	6.093	6.593
Benzo(a)pyrene	8.430	10.390	8.277	7.593	9.707
Indeno(1,2,3-cd)pyrene	5.683	8.705	4.917	2.597	6.103
Dibenz(a,h)anthracene	8.397	4.025	1.867	2.780	3.400
Benzo(g,h,i)perylene	11.973	9.680	13.893	11.530	10.080

4.3 ATR-FTIR analysis of tarmat samples

ATR-FTIR analysis was carried out for tarmat samples collected from 15 locations along the Qatar coast. The characteristic peaks for aliphatic hydrocarbons are related to stretching, bending and rocking vibrations at 3100–2800 cm^{-1}, 1460 cm^{-1}, 1377 cm^{-1} and 720 cm^{-1}, respectively (Fig. 4.6). Specific peaks for aromatic hydrocarbons are found at 1600 cm^{-1} and 900–700 cm^{-1}. The peaks at 1800–1600 cm^{-}

[1] indicate the presence of oxygenated functions. The ATR-FTIR characteristic peaks for various functional groups have been assigned and reported in Table 4.

Table 4. Interpretation of Peaks Observed in the Tar Samples

Peaks (cm^{-1})	Interpretations	References
3390	Broad peak. Hydroxy group, H-bonded. OH stretch	(Derrick et al., 1999, Mayo et al., 2003, Stuart, 2004, Silverstein et al., 2005, Field et al., 2008, Pavia et al., 2009. Coates et al., nd)
2953	Asymmetrical stretching of C-H bond in CH3 and stretching of C-H bond in alkanes	
2852	Symmetrical stretching of C-H bond in CH3 and asymmetrical stretching of C-H bond in CH2	
1700	Stretching of C=O in secondary amides	
1600	Stretching of C=C in aromatic rings	
1460	Symmetrical bending of C-H in CH2 , asymmetrical bending of C–H in CH3 and asymmetrical stretching of C=C bond in aromatic rings	
1376	Symmetrical bending of C–H bond in CH3	
1030	Stretching of S=O bond in sulfoxides	
864	Out of plane bending of C–H bond in aromatic compounds	
814	Out of plane bending of C–H bond in aromatic compounds	
744	Out of plane bending of C–H bond in aromatic compounds	
724	Out of plane bending of C–H bond in aromatic compounds and bending (rocking type) of C–H in CH2	

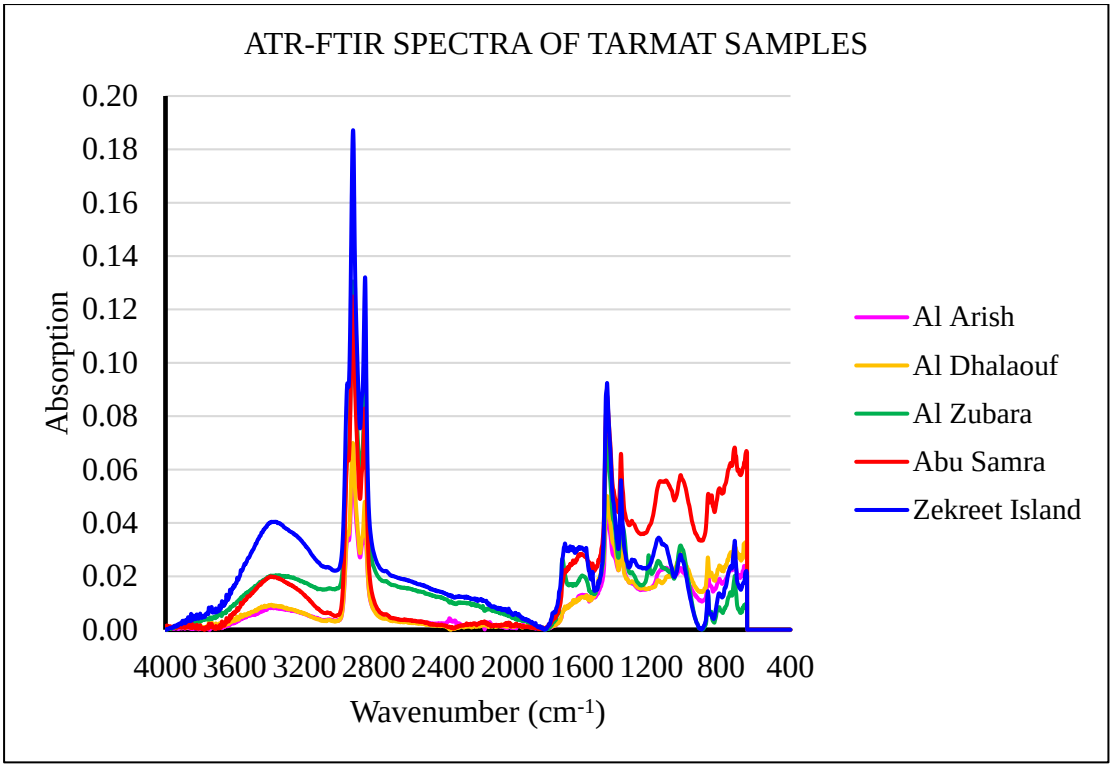

Figure 7. Representative ATR- FTIR spectra of tarmat samples

Peaks in the region between wavenumber 3000 and 2850cm^{-1} tend to overlap with absorption peaks for different groups, whereas peaks observed between 1750 and 650cm^{-1} including the aromatic ring and long chain aliphatic hydrocarbon vibration show variations in all the 15 tarmat samples which could be due to the difference in the type and composition of the tarmats (Fig 7).

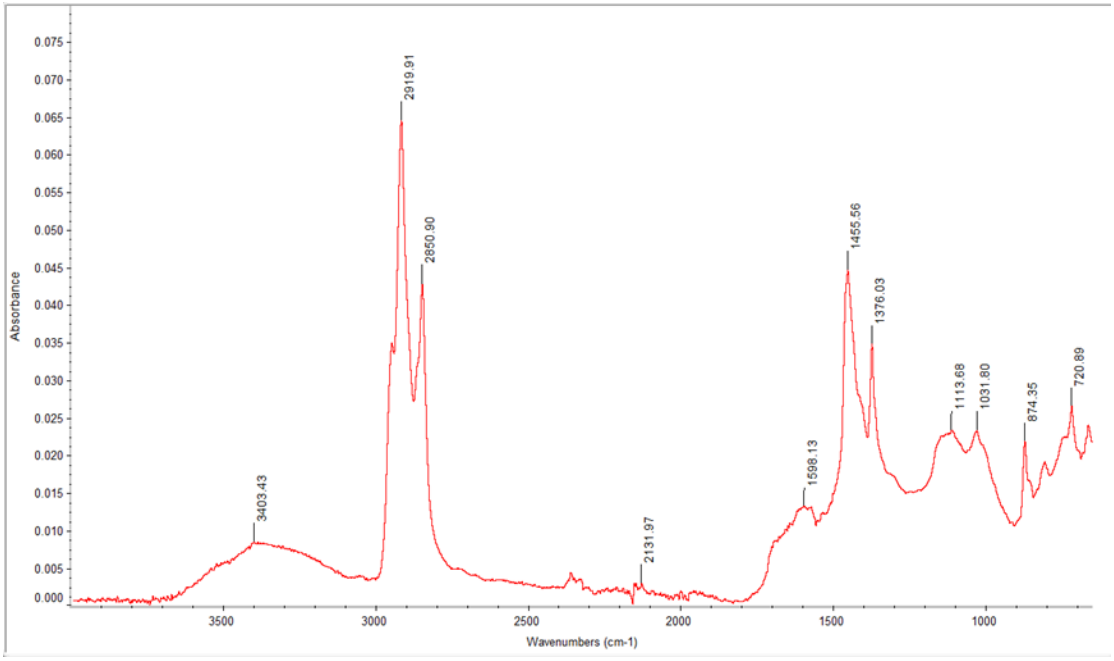

Figure 8. ATR -FTIR spectra of tarmat samples from Al Arish

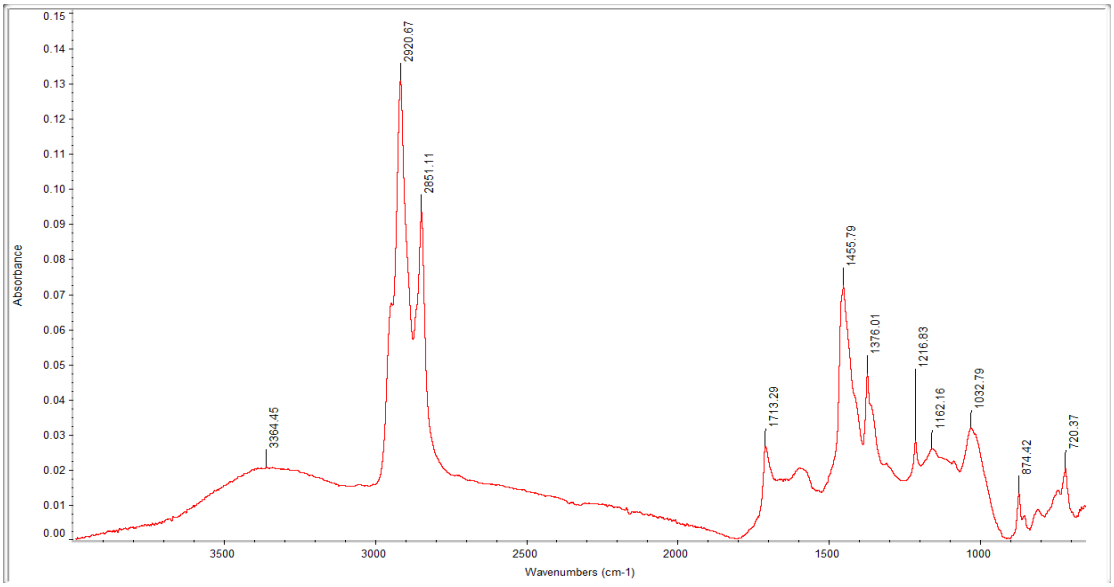

Figure 9. ATR -FTIR spectra of tarmat sample from Al Zubara

As seen in the above two spectra (Fig 8 & 9), all the samples show similar trends of peaks with notable peaks included within the wavenumbers 2919cm^{-1}, 2850cm^{-1},

1460cm^{-1}, 1376cm^{-1}, 1030cm^{-1}, 864cm^{-1} and 720cm^{-1}. Most notable larger peak areas for all samples are at 2919cm^{-1} and 2850cm^{-1}, indicating C-H stretching in alkanes. Smaller peak areas of C=O at 1700 cm^{-1} and C=C at 1600 cm^{-1} peaks were observed for all the samples, indicating less photo-oxidation/degradation and lower percentage of aromatic molecules, respectively. Small peaks were also observed in the region between 890 and 730cm^{-1}, indicating resins and asphaltenes in the samples. Oxygen containing functional groups of O-H stretching at 3390 cm^{-1} with smaller peak areas of 0.0001-0.0003 was not detectable in most of the samples. Stretching of bond S=O sulfoxides at 1030cm^{-1} were also observed as relatively weak stretch.

4.4 Calculation of Spectrometric Indices

The following functional and structural indices have been calculated for all the 15 samples using peak areas, which can be used to determine the chemical composition of the compound. Aliphatic index is found to be higher than aromatic index for all the samples along the Qatar Coast. Tarmats along the western coast showed higher aliphatic index, whereas tarmat along the eastern coast showed higher aromatic index (Table 5).

Table 5. Spectrometric Indices for Tarmat Samples along the Qatar Coast.

	Aliphatic Index	Aromatic Index	Long Chain Index	Substitution 1 Index	Substitution 2 Index	Aliphatic /Aromatic
Western coast	1.1921	0.0084	0.0769	0.9512	0.4262	426.1550
Southern Coast	0.1706	0.0008	0.0003	0.5993	0.5592	202.8021
Eastern Coast	0.5498	1.1608	0.1831	1.0959	0.4499	96.4313
Northern Coast	1.4509	0.4427	0.6534	2.8758	0.7817	50.3765

4.5 Determination of aromatic compounds from FTIR.

The Aromatic Index, Substitution 1 index and Substitution 2 index represent, presence of all aromatic compounds in the sample. Condensation degree of aromatic compounds is indicated by the substitution Index. Aromatic index against substitution Indices is plotted in Fig 10. Substitution Index is higher for the samples collected from the eastern and northern coasts of Qatar compared to the western and southern coast samples. This indicates that western and southern coast samples have weakly condensed aromatic ring, whereas the eastern and northern samples are characterized by greater aromatic character with strongly condensed structure.

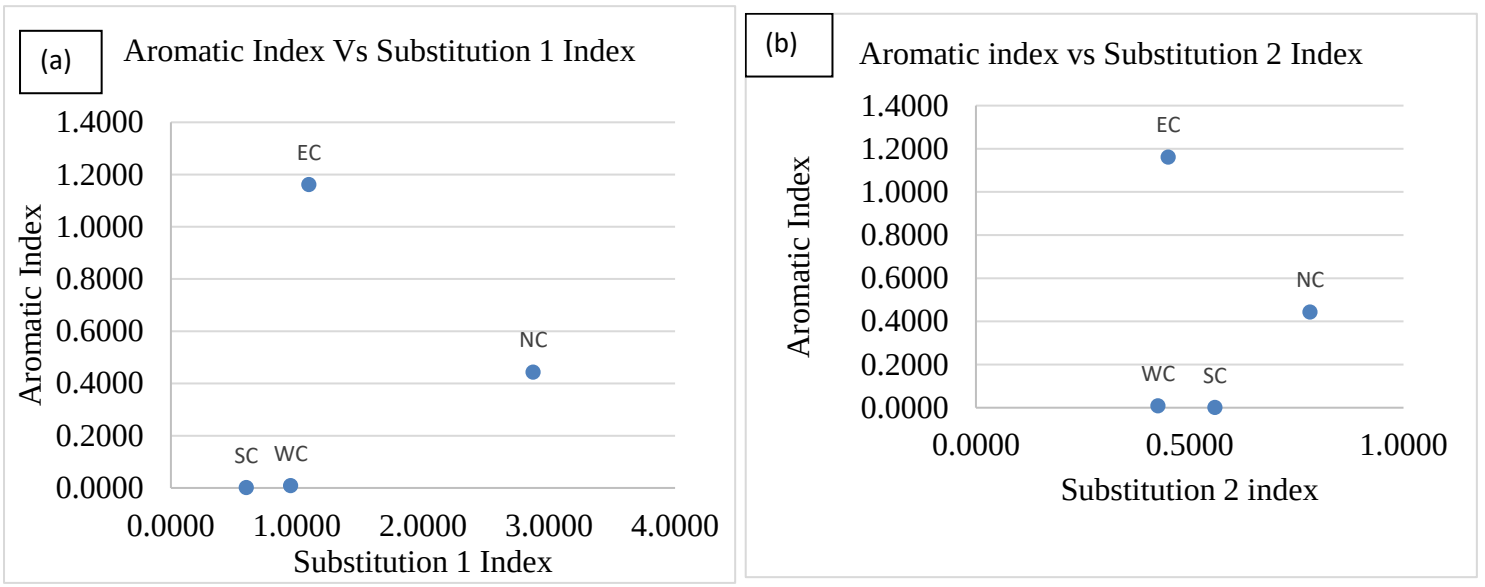

Figure 10. FTIR aromaticity index vs substitution 1 index (a) and substitution 2 index (b).

4.6 Determination of Aliphatic Compounds

Long Chain Index and Aliphatic Index are used to represent all aliphatic compounds in the sample (Fig. 11). It is found that northern and western coasts show higher quantity of long chain aliphatic compounds, compared to eastern coast and southern coast samples.

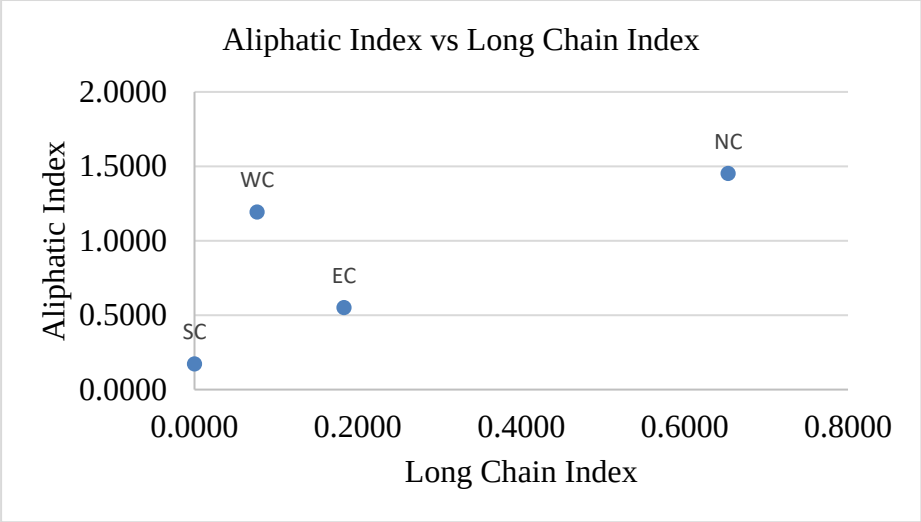

Figure 11. FTIR aliphatic index vs long chain index.

4.7 Maturation Degree of oil deduced from FTIR

Maturation degree of oil residues can be determined by finding the ratios of aliphatic compounds vs aromatic compounds. Higher the ratio of oil residues, higher will be the maturation degree. Fig.12 clearly indicates higher order of maturation for tarmats along the western coast; southern, eastern and northern coasts are in the decreasing order of maturation.

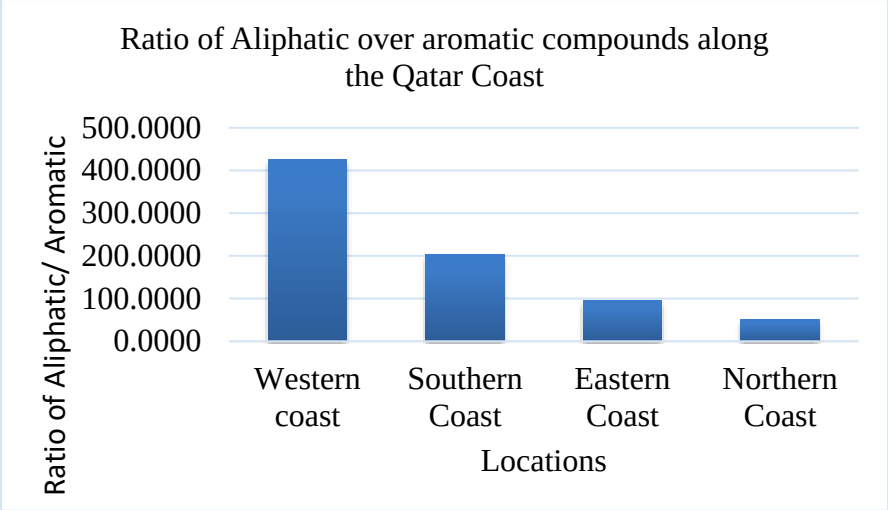

Figure 12. Ratio of aliphatic compounds over aromatic compounds for determining the maturation of oil

4.8 Assessing the concentration of DNA in tarmat samples

Most of the samples showed average A260/A280 ratios of 1.7 – 1.8 through Nanodrop spectrophotometer. A260/A280 values greater than 1.8 are actually considered suitable for analysis. Southern Fuwairit samples along the eastern coast showed lower absorbance values (1.18), whereas Ras Rakan samples showed higher value (1.9). (Fig 13).

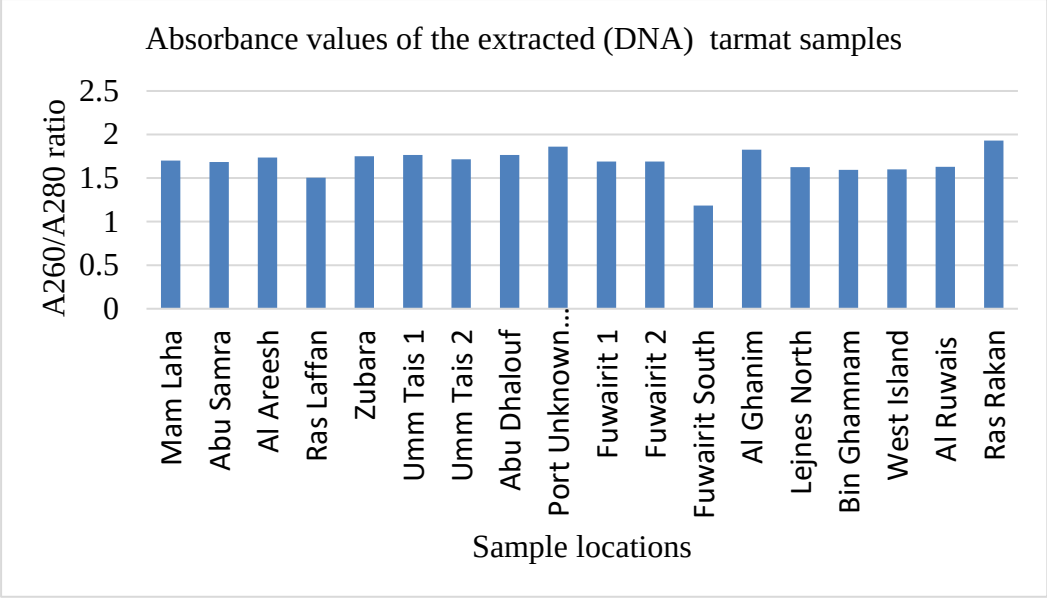

Figure 13. Absorbance values at A260/A280 ratios of extracted DNA in tarmat samples

4.9 Quality Control Data for Genome Libraries

After performing PCR, size of the enriched PCR fragments were analyzed by running on bioanalyzer using DNA 1000 chip from Humanizing Genomics Macrogen (South Korea). This is a highly sensitive technique which is considered as an essential step prior to sequencing, and samples between medium to upper range concentration only can be used for successful run. All the 18 samples have passed the Quality Control test, with size and concentration ranging from 596 to 604 bp and 40.35-60.07ng/µl, respectively (Table 6).

Table 6 Library QC result of DNA in Tarmat Samples

Library Name	Conc. (ng/µl)	Size (bp)	Result
Mam_Laha	50.64	603	Pass
Abu_Samra	51.48	596	Pass
Al_Areesh	50.99	600	Pass
Ras_Laffann	40.35	604	Pass
Zubara_S1	47.59	602	Pass
UmmTais_4	47.51	606	Pass
UmmTais_1	43.64	608	Pass
Abu_Dhalouf	39.86	600	Pass
Port_Island	60.07	609	Pass
Fuwairit_S1	46.33	602	Pass
Fuwairit_S2	43.73	602	Pass
Fuwairit_So2	45.47	598	Pass
Al_Ghariyah	52.76	603	Pass
Lejnail	54.47	602	Pass
Bin_Ghamnam	50.93	602	Pass
West_Island	50.35	598	Pass
Al_Ruwais	48.02	599	Pass
Ras_Rakan	51.35	604	Pass

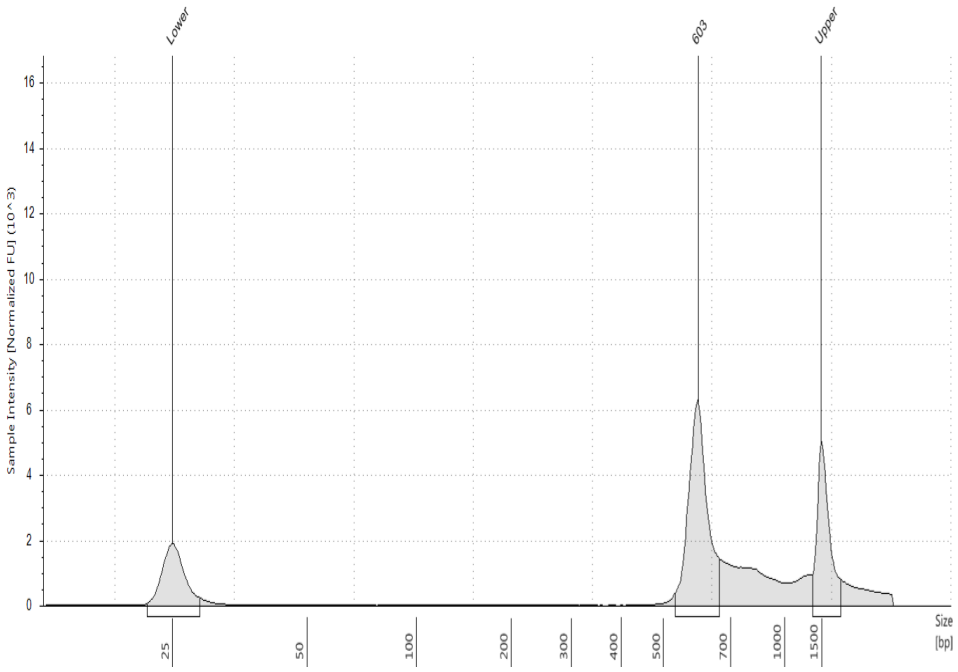

Figure 14. Electropherogram of libraries of Mam Laha Tarmat sample from the Agilent D1000 TapeStation system shows a peak at 603 bp.

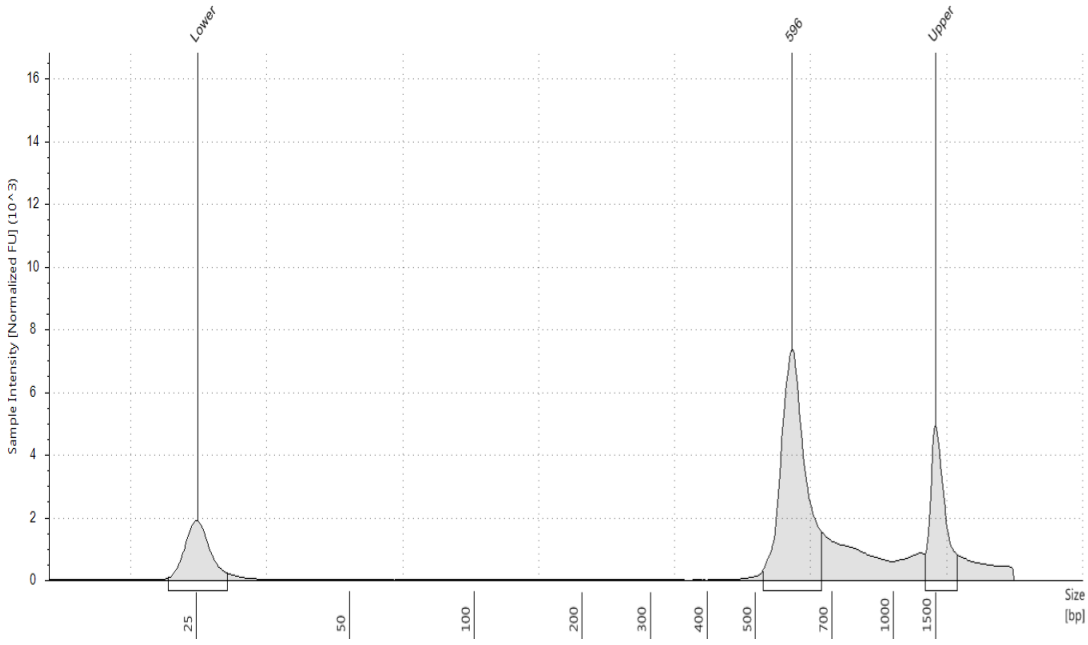

Figure 15. Electropherogram of libraries of Ras Laffan Tarmat sample from the Agilent D1000 TapeStation system shows a peak at 604bp.

4.10 Raw Data Statistics of Sequencing

4.10.1 Total Read Bases and Total Reads

The Illumina sequencer generates raw images utilizing sequencing control software for system control and base calling through an integrated primary analysis software called RTA (Real Time Analysis). The BCL (base calls) binary is converted into FASTQ utilizing illumine package bcl2fastq. The total number of bases, reads, GC (%), AT% Q20 (%), Q20 %nd Q30 (%) are calculated for the 18 samples. For example, in Abu Samra sample, 196580 reads are produced, and total read bases are 59.1M bp. This indicates that 59.1M bp are sequenced and total reads value refers to the sum of reads 1 and 2 for Illumina paired end sequencing. Adapters are not trimmed away from reads. Based on the statistical results, the tarmat sample preparation for metagenomics analysis showed Q20% values more than 90% for all samples. Therefore, the reliability of metagenomics results are very high.

Table 7. Summary of Total Read Bases and Total Reads Statistics of Libraries.

Sample ID	Total read bases (bp)	Total reads	Q20%
Mam_Laha	62,383,454	207,254	91.78
Abu_Samra	59,170,580	196,580	92.07
Al_Areesh	50,628,200	168,200	91.60
Ras_Laffan	55,095,040	183,040	90.98
Zubara_S1	66,880,394	222,194	92.37
UmmTais_4	53,688,768	178,368	92.67
UmmTais_1	60,257,792	200,192	92.12
Abu_Dhalouf	68,367,334	227,134	91.92
Port_Island	43,894,830	145,830	91.63
Fuwairit_S1	57,698,690	191,690	92.39
Fuwairit_S2	57,830,528	192,128	92.46
Fuwairit_So2	43,880,382	145,782	91.47
Al_Ghanim	55,134,772	183,172	91.36
Lejnes_North	55,486,340	184,340	91.66
Bin_Ghamnam2	55,677,776	184,976	92.08
West_Island	60,403,476	200,676	91.55
Al_Ruwais	57,462,706	190,906	92.06
Ras_Rakan_1	56,212,954	186,754	92.64

4.10.2: GC and AT content percentage

GC content is commonly used as marker in bacterial systematics and bacterial species show high variations of GC content in their genome. The raw data for GC content (Fig. 16) is estimated without trimming the adapters from the ends. GC content for all the samples ranged between 50 and 60%, which can be considered as moderate value. For example, in Mam Laha, the GC content is 55.91%, which is in between

moderate to high GC content.

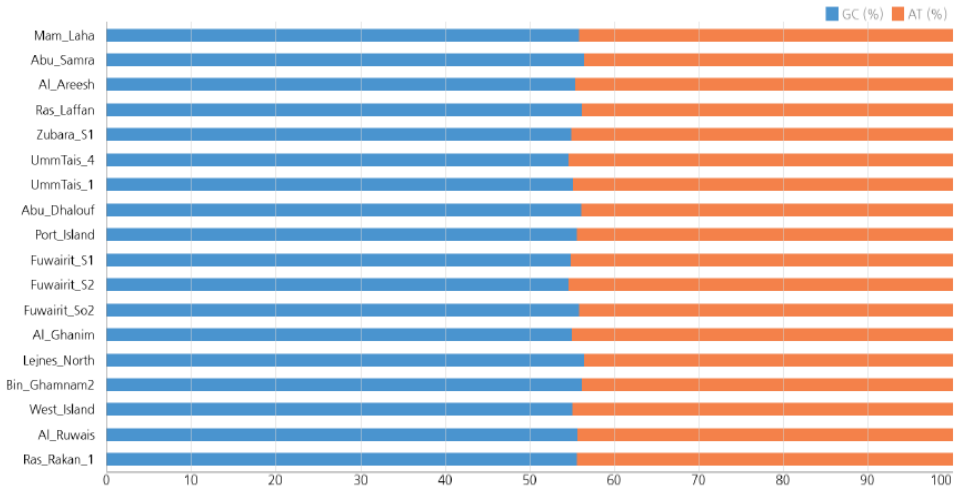

Figure 16. GC/AT content of raw data

4.11 Bioinformatics Result

4.11.1 A brief summary of the diversity of tarball associated bacteria in tarmats.

Taxonomic classification has been grouped into Amplicon sequence variants (ASV) / Operational Taxonomic Units (OTUs), ordered by reading abundance, in order to evaluate the connectedness of the sequence variants. There have been a total of 9172 OTUs recorded after the removal of singletons. (Table 8). This segment includes the top ten relative abundance bar plots for phylum, class, order, family, genus of bacterial communities in tarmats.

Table 8. Summary of Reads and Operational taxonomy units (OTUs)

Read description	Details
Total Reads	903256
Total OTUs Picked	9172
Total OTUs after Singleton removal	8927

The OTUs are used to generate refraction curves, to calculate the abundance as well as estimating the diversity indices. Below table (Table 9) reports the OTU classification at phylum, order, class family and genus level in which only top 10 categories are reported. For further reporting the data , phylum and genus levels are used. A total of 10 phyla were recorded among the eighteen tarmat samples studies. Phylum proteobacteria have recorded higher level of genera category compared to other phyla. Actinobacteria, Bacteriodetes, Chloroflexi , Cyanobacteria, Deferribacteres, Plantomycetes, Proteobacteria, Synergistetes , Thermodesulfobacteria and Thermotogae were the top 10 phyla recorded.

Table 9. Taxonomy Classsification of the OTU's at Phylum, Order, Family and Genus level. Only top 10 categories are shown

Phylum	Class	Order	Family	Genus
Actinobacteria	KIST JJY010			
Bacteriodetes	Bacteriodia	Bacteriodales	Marinilabiliacea	*Anaerophaga*
		Rhodothermales	Rhodothermacea	
Chloroflexi	Anaerolinae			
Cyanobacteria	Oxyphotobacteria	Nostocales		
Deferribacteres	Deferribacteres	Deferribacteriales	Deferribcateriacea	
Plantomycetes	Phycisphearae			
Proteobacteria	Gammaproteobacteria	Chromatiales, Oceanospirillales, Rhodobacterales, Sphingomonodales, Alteromondales	Rhodobacteracea, Sphingomonodacea, Alteromonodacea, Acidithiobacillacea, Ectothiorhodospiracea	*Alkalilimnicola Alcanivorax, Defluviococcus, Parvularcula, KCM-B-112, Marinobacter, Salinimonas*
Synergistetes	Synergistia			
Thermodesulfobacteria	Thermodesulfobacteria		Thermodesulfobacteriacea	*Thermodesulfobacterium*
Thermotogae	Thermotogae	Petrotogales	Petrotogacea	Petrotoga

4.11.2 Relative abundance of Tarmat associated Bacteria at Genus and Phylum levels.

As mentioned earlier, a total of 10 phyla were recorded from the studied tarmat sites. Below figures (Fig 17 - 18) is used to interpret the abundance of bacteria in these sites.

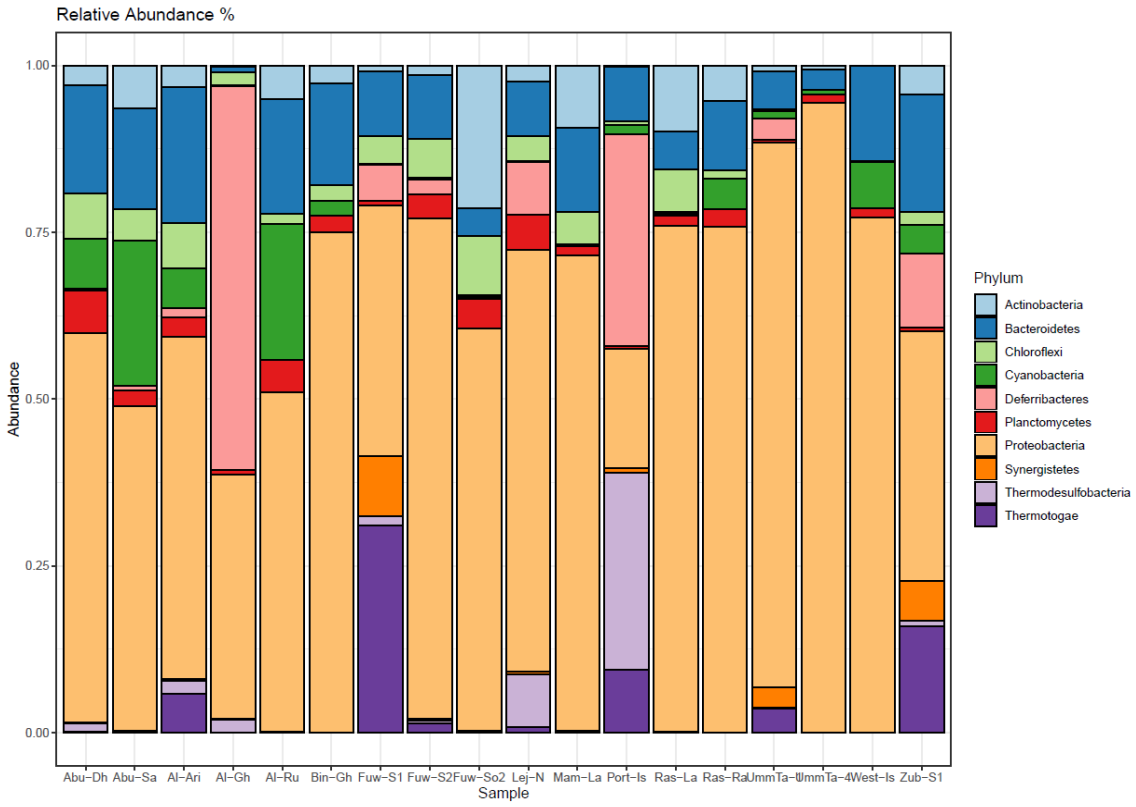

Figure 17. Relative abundance of top 10 enriched phylum in the tar-mat of the study area

Phylum proteobacteria is the most recorded abundant phylum (approx. 50- 90%) in all the samples except Al Ghanim and Port Island whereas the relative abundance of the remaining 9 phyla showed variations among all the sites. The other important groups of phyla include Bcteriodetes, Chloroflexi and Actinobacteria. Phyla such as

Thermotoage, Thermodesulfobacteria and Deferribacteres were present in higher abundance in only few of the sites. On the other side, phylum Synergistetes were recorded only in Fuwairit, Port Island, Umm Tais and Zubara.

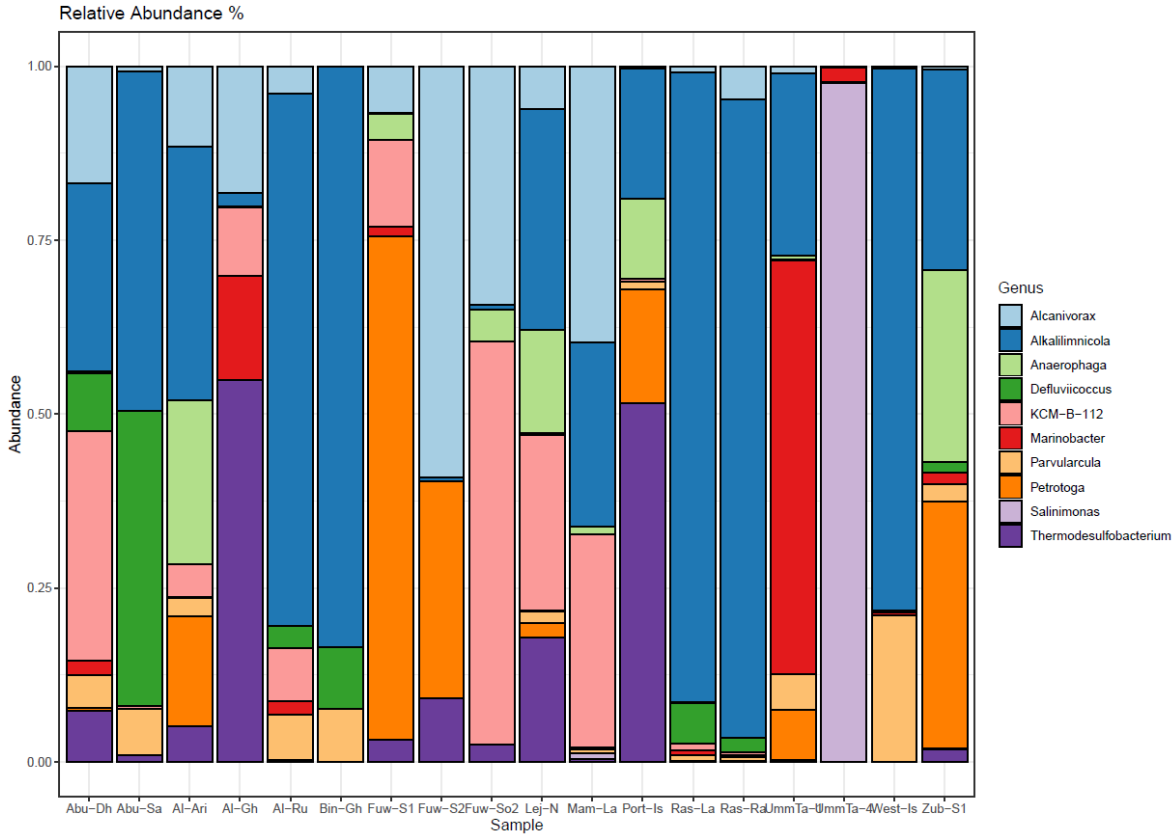

Figure 18. Relative abundance of top 10 enriched genus in the tar-mat of the study area.

The above figure (Fig 18) shows the relative abundance of top 10 enriches genes of selected ASVs. From the figure, it clearly depicts that genera community has shown variations among the studied tarmat sites . The below table (Table 11) shows the

classification of the genera into their phylum and class levels of the top 10 abundant ASVs.

Table 10. Taxonomy Classification of the OTU at phylum , genus and class levels of selected abundant ASVs.

Selected ASV	Phylum	Class	Genus
ASV_1	Proteobacteria	Gammaproteobacteria	Alkalimnicola
ASV_4	Thermodesulfobacteria	Thermodesulfobacteria	Thermodesulfobacteria
ASV_5	Proteobacteria	Gammaproteobacteria	Alcanivorax
ASV_8	Proteobacteria	Alphaproteobacteria	KCM-B-112
ASV_12	Proteobacteria	Gammaproteobacteria	Salimonas
ASV_10	Thermotogae	Thermotogae	Petrotoga
ASV_16	Proteobacteria	Alphaproteobacteria	Parvularcula
ASV_17	Proteobacteria	Gammaproteobacteria	Marinobacter
ASV_18	Bacteriodetes	Bacteriodia	Anaerophaga
ASV_26	Proteopbacteria	Alphaproteobacteria	Defluvicoccus

Alkalimnicola, Alkanivorax, KCM-B112 and Defluvicoccus were the most important genera found in most of the sites. All of these groups are from the Phyla Proteobacteria . There are some unique observations recorded among the genera. For Example, *Salimonas* of Proteobacteria phyla were only observed in tarmats of Umm Tais sample. *Thermodesulfobacterium* were observed in Al Ruwais and Port Island with a relative

abundance of 50%. Other important genera groups include *Anaerophaga and Marinobacter.*

4.11.2 Alpha Diversity and Rarefaction Curve

The alpha microbial diversity within all samples is determined through Shannon, Chao1, Simpson and observed species metrices using phyloseq R package. The chao1 metric estimates the species richness, Shannon estimates species abundance and Simpson estimates species dominance in the samples (Table 11). The observed species metric is only the count of unique OTUs identified in the sample. From the observed metric count, higher abundance of OTUs were observed among northern coast from Abu Dhaluf and Al Ruwais and Al Zubara tarmat samples from the eastern coast. Shannon Index value ranged between 2.4 (Port Island) , 2.94 (Ras Laffan) to 6.24 (Abu Dhalouf). Higher Shannon Index values are observed for northern coast, followed by west, east and south coasts. Similarly, high species richness Chao1 is estimated for Abu Dhalouf (1913) and Al Ruwais (1076) among the northern coasts whereas and for Zubara (1028) from the western side. Similar to Shannon Index , lower value for Chao1 is estimated for Port Island. Inverse simpson index values were higher in Abu Dhalouf tarmat samples, whereas lower value is observed for Ras Laffan and Port Island samples.

Table 11. Alpha Diversity Indices of the Tarmat Sample.

	Observed	Chao1	Shannon	InvSimpson
Abu.Dhaluf	1913	1917.577	6.244576	114.395404
Al.Ruwais	1076	1077.258	5.221595	29.27702433
Bin.Ghamnam	480	481.2353	3.573704	6.334377575
Lej.Nail	732	732.3061	4.351133	23.25614327
Mam.Laha	520	520.375	4.372819	28.07936221
Port.Island	257	257.2857	2.49745	5.166215635
Ras.Rakan	705	706.6071	3.346085	4.420515932
UmmTais.1	665	665.3846	4.419094	21.54204755
UmmTais.4	447	447.8077	4.143308	19.51460762
Al.Ghariyah	480	480	3.316468	7.901866379
Fuwairit.S1	564	564.2941	4.558021	35.74079696
Fuwairit.S2	890	891.0185	4.678863	23.34513731
Fuwairit.So2	463	463.1875	4.138817	17.49651053
Ras.Laffan	535	535.1935	2.943431	3.701119448
Al.Arish	717	717.3333	4.894548	36.83984065
West.Island	638	638.3571	3.507469	6.428347292
Zub.S1	1028	1028.71	4.727162	29.50847188
Abu.Sa	676	676.5676	4.449558	21.6202625

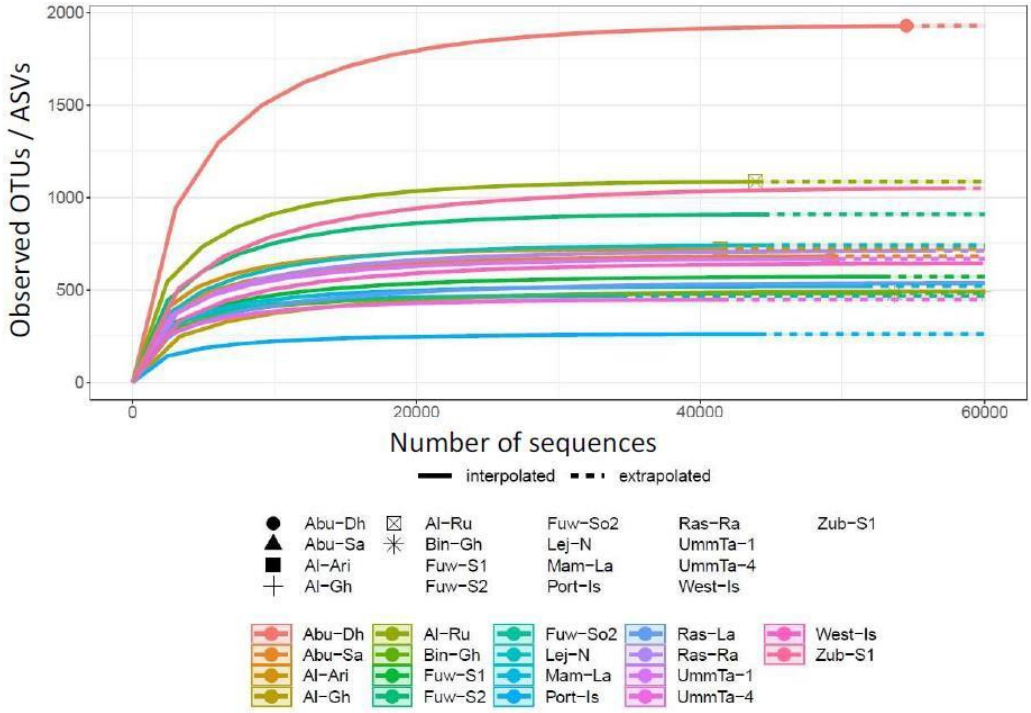

Figure 19. Rarefactions curve of the tar-mat sample from the various sampling locations

Rarefactions curve is plotted between observed OTUs/ASVs and number of sequence, where each curve is extrapolated to 60000. This nonparametric sequence curve is used to compare the OTU richness among the tarmat sites. Rarefaction curves of all the samples showed the curve plateaus which indicated that in both the samples the species richness had not approached completeness. In the plot above Abu Dhaluf with higher rarefaction curve is the more diverse, as the number of different species (OTUs) increase more than in other samples, with increasing number of sequences. In contrast the Port Island sample is the less diverse, as the number of species does not increase very much regardless of the number of sequences that have analyzed.

4.11.3 Beta Diversity between Tarmat samples

Beta diversity represents the explicit comparison of microbial communities based on their comparison. In this study the comparison is performed between all the samples. First, we generate the distance matrix using UniFrac approach. UniFrac takes input phylogenetic information and provides comparison between microbial communities. The unweighted UniFrac approach was considered for sequence abundance when comparing microbial diversity. Principal component analysis (PCoA) at Phylum and genus level was also performed on the sample.

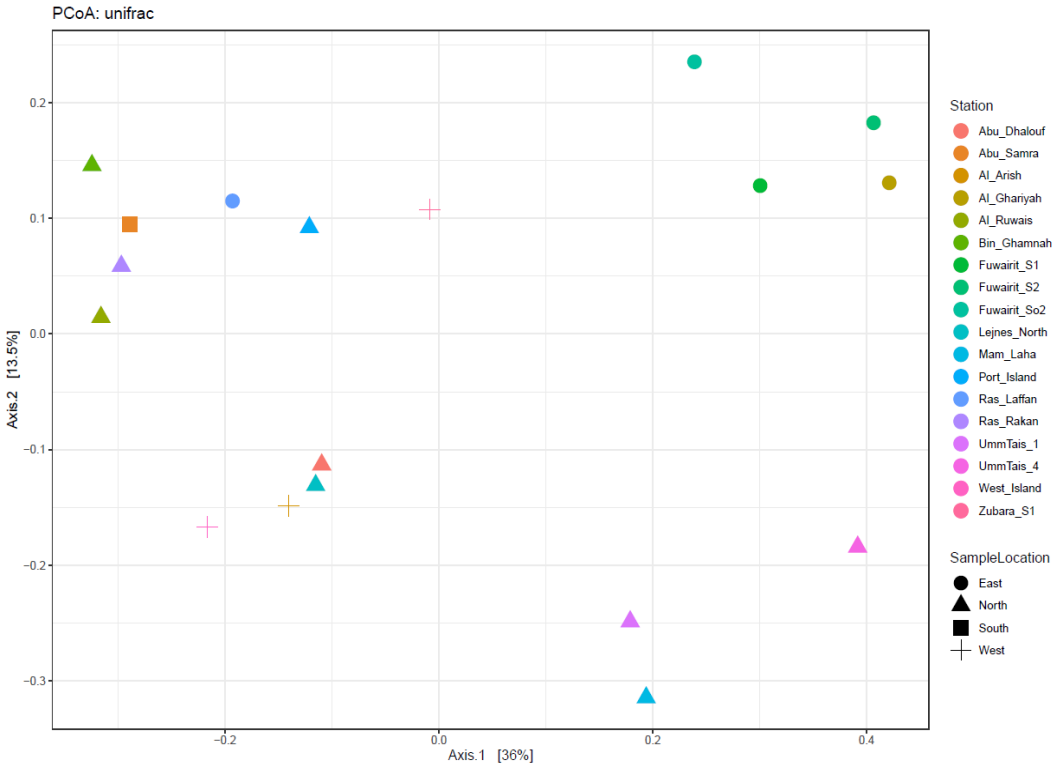

Figure 20. Principal component analysis (distance based on unifrac) of various samples of tar- mat in the coastal line of Qatar.

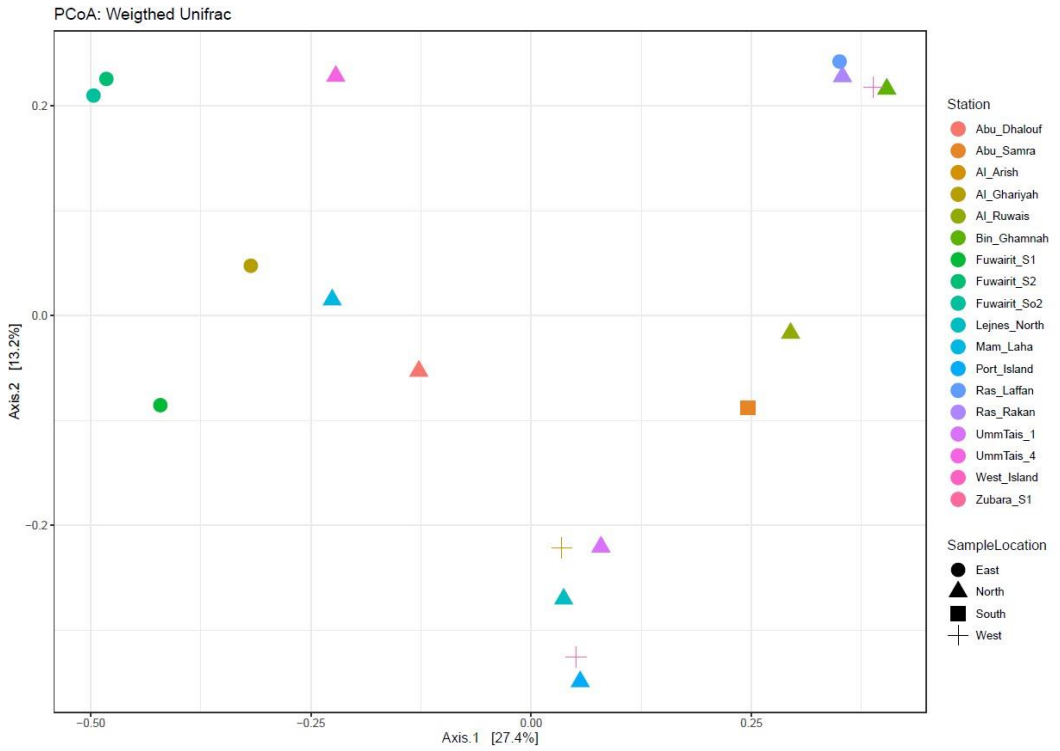

Figure 21. Principal component analysis (distance based on weighted unifrac) of various samples of tar-mat in the coastal line of Qatar.

Here, we have used both weighted unifrac (Fig 21) and unweighted or original unifrac (Fig 20) as qualitative and quantitative measures of beta diversity respectively. Unweighted UniFrac approach was used to compare the abundance of sequence diversity among the microbial communities. Fig 20 dustinguish eastern coast samples (except the Ras Laffan samples) from other coasts. This clearly indicates that phylogenetic relation among the microbial communities among the eastern sample are more related and are clearly distinguished from the other locations, as these locations are clustered together. These results confirms with the obtained alpha diversity indices as well. Fig 21 which explains the weighted unifrac approach takes input phylogenetic information and provides comparison between microbial communities. It is highly

difficult to distinguish the coasts into a specific clusters from this plot as most of the locations are scattered all over the plot. However two of the Fuwairit samples among the eastern coasts are clustered together. Microbial communities observed among the Lejnail, Mam Laha and Port Island of northern coasts are as well clustered with tarmat samples of Al Arish and Zekreet along the western coast.

CHAPTER 5: DISCUSSION

Qatar, a fast developing country with increasing industrial, technological and educational advances, relies heavily on marine resources, which put a lot of attention on marine pollution to take into deep consideration. Heavily relying on desalinated water, booming fisheries along with coastal tourism are currently impacted by marine pollution. Out of 1955 documented species of fauna and flora in Qatar, 955 belong to marine species. Despite hyper-arid, hypersaline and thermo-variable conditions, these species exhibit strong adaptation characteristics with an increase in their biodiversity (Macdonald, 2007). This unusual adaptations to sever environments further indicates an improvement and advancement in biotechnological opportunities. In the Arabian Gulf, oil-utilizing bacterial associated with fish have the potential for cleaning oily waters (Radwan et al., 2007). This is believed to arise from natural oil seeps in the Gulf area, resulting in a fairly large amount of hydrocarbons in the water creating preferential demand for hydrocarbon usage or sequestration. These biological tools may potentially be used to mitigate oil spills.

A study focusing on the marine tar contamination in Qatar has great significance and importance to understand the state of oil pollution along the Qatar coast and eventually to adopt both environmental and sustainable friendly remediation strategies. The major objective of this study is to understand variations in bacterial communities in tarmats deposited along the coast of Qatar and finding suitable ways to link the distributions of these bacterial populations to the chemical composition of tarmats. As mentioned earlier, such a kind of study has not been conducted before. Though many oil spill related studies were carried out in the Arabian Gulf after the Gulf War spill in 1991, less interest was reflected in the literature since the 21st century. But, in recent years, there have been a resurgence in tar residue related studies in the Arabian Gulf.

Al Kaabi et al. (2017) demonstrated the presence of huge amount of tar residues in Zubara, which has great national heritage significance to Qatar. It is also important to note that most of the studies regarding microbial population were solely focused on the isolation and culturing of hydrocarbon-degrading bacteria from oil-polluted soils. Understanding the characteristics and metabolic activities of these populations especially in the Gulf region since the weathering process is highly accentuated in the arid climate, could be of great importance to oil pollution studies. In addition to this, certain biotic and abiotic factors help the organisms to adapt such harsh conditions for a suitable bioremediation strategy (Albokari et al., 2015).

5.1 Tarmat Deposition along the Qatar Coast

For the present study, tarmat samples were collected from 16 locations along the Qatar coast, namely, Abu Samra , Al Arish, Al Zubara, Zekreet, Ras Laffan, Al Ghariyah, Fuwairit, Umm Tais, Ras Rakan, Bin Ghamnam, Al Ruwais, Mam Laha, Ruwais Port Island and Abu Dhalouf. Different degrees of weathering from large and heavily weathered to fresh sticky oil have been observed, indicating that beaches and islands had constant exposure to oil pollution. Most of the coasts were coated with tarmat mixed with moderate to high quantities of seaweed, sand, barnacles, shells and marine litter. Al Madfa (1999) recorded higher tar concentration in northwest (723g/m) and north (620g/m) coast of Qatar. The lower tar concentration values were found in the east (150g/m) and west (304g/m) coast of Qatar. Al-Zubara, Al-Arish, Al Ghariayh and Fuwayrit are the most impacted beaches by Nowruz and Gulf War oil spills. The northwestern and northern part of Qatar are heavily exposed to high traffic activity. High tar levels in Ras Laffan could be due to the influence of current flowing from Hormuz (Al Madfa, 1999) and offshore oil-related activities. Most of the northern shores along the Qatar coastlines are contaminated with more tar residues than other

parts. Tar deposition along the east coast of Qatar is significantly less compared to northern beaches. Three decades ago, Aboul Dahab (1994) assessed the state of oil pollution in Qatar through the estimation of tar deposits along the beaches and petroleum hydrocarbons in waters. According to previous studies, Al Arish and Al Zubara beaches have huge tar deposition of more than 5000 gm^{-1} months and were difficult to assess the concentration in these areas. Northwest parts of Qatar are heavily polluted as these regions are prone to both accidental and chronic oil spillages and these coasts receive the first heavy coating of oil during any oil spill event. Less tar deposition on northeastern sides is mainly due to the higher wave energy that encourages a self-cleaning process as well as protective nature of the coast to the arrival/deposition of spill/ oil residues. The peninsula's southwest coast is much less vulnerable to the direct impact of tanker traffic. In this area, there are no oil terminals or ports and just intermittent maritime traffic. Furthermore, the absence of offshore oil fields reduces the risk of oil pollution.

5.2 Chemical Quantification and Qualitative Degradation of TPH and PAH in Tarmats using GC and FTIR analysis

5.2.1 GC Analysis

Gas Chromatography is the most common method, which has been successfully developed by Ehrhardt and Blumer (1972) to quantify the chemical composition of tar and oil residues. With information from GC analysis about the relative quantities of components in tar, it is possible to conclude the source of tar through comparison of several suspected source oils. Tar residues were further characterized by several methods of detection, including GC coupled with FID, AES and MS and several extraction techniques for preparation of samples for analysis. In the present study, the PAHs and TPH concentrations in tarmat samples from the select locations (Abu Samra,

Abu Dhalouf, Ras Laffan, Ras Rakan, and Al Arish) along the Qatar coast were estimated using GC-MS and GC-FID, respectively. The extraction was carried out using a normal solid-phase extraction cartridge, which made the extraction procedure less time-consuming.

Abu Samra along the southern coast and Abu Dhalouf along the northern coast showed higher levels of TPH (Fig 5). TPH was not detected in tarmat samples from Ras Laffan and Al Arish. Abu Samra and Abu Dhalouf tarmat samples had higher PAH concentration, like TPH values, among the analyzed samples. HMW PAHs such as chrysene, benzo[a]pyrene, dibenz[a,h]anthracene and Benzo(g,h, i)perylene were present in higher concentration and LMW PAHs such as fluorene and phenanthrene were completely absent. Among the LMW PAHs, naphthalene showed higher concentration, ranging from 8 to 10ppb in all locations. Among HMW PAHs, benzo(a) anthracene showed lower concentration ranging from 0.6 to 2.1 ppb (Fig 6). These variations can correspond to their sources and the physical and chemical properties of the PAHs (Ranjbar Jafarabadi et al., 2017a; Rahmanpoor et al., 2013).

It is quite interesting to note that Ras Laffan samples have shown no detectable levels for TPH and also low levels of PAH concentration. But, compared to other areas, tarmat samples from Ras Laffan showed higher levels of LMW PAHs, which is probably because this area is subject to a wide range of petrochemical activities. In general, beached tar above the high tide can be highly exposed to photodegradation along with biodegradation process. PAH components can be photooxidized directly by absorbing UV radiation (Lee, 2003), and their toxicity has been found to increase after the process (Maki et al., 2001; Lee, 2003; Barron et al., 2005).

The results from our study are relatively comparable to a previous study that determined the concentration of petroleum hydrocarbons along the Qatari coastline

(Aboul Dahab,1994). This study demonstrated that petroleum hydrocarbons heavily contaminate all sites along the coastline, and concentration is higher along the north-western side. Considering the regular occurrence of accidental and persistent oil spills in the northwest of Qatar along with wind conditions, heavy oil emission is predicted in those areas. On the other hand, northeastern and eastern shores are more vulnerable to waves that may intensify the degradation mechanism and therefore reflect a lower level of pollution. Rushdi et al. (2017) quantified the PAH concentration (mean value 0.09 ± 0.15%) in sediment samples from Qatar coast. Among the sampled locations, Ras Laffan has the highest concentration of benzo[ghi]fluoranthene and benz[a]anthracene. However, the concentration of PAHs in Doha is higher than those found in Ras Laffan, probably because of the traffic of several boats and vessels.

5.2.2 ATR-FTIR Analysis

The most important and versatile analytical tool that has considered for successful identification of petroleum compounds is Gas Chromatography, since it can yield highly sensitive and accurate results, coupling with other analytical methods such as GC-MS and GC–FID. But, most of these techniques are tedious and time consuming, and required to follow variety of sample preparation methods. However, FTIR techniques have evolved in developing a non-expensive and highly simple and rapid for general compositional analysis of oil residues. Along with chemometrics and statistical studies, these techniques provide a fast determination of different chemical parameters in crude oil (Aske et al., 2001; Hannisdal et al., 2005) and have been widely applied in various industries.

For the present study, the ATR-FTIR spectra were recorded for tarmat samples from 15 locations to understand the qualitative degradation. To understand the chemical composition of tarmats, various spectral Indices were calculated. Calculation of these

indices is highly reliable as it takes into account several vibrations, which are occurring simultaneously for the same type (Permanyer et al., 2001). Calculated indices include an aliphatic and long-chain index for representing the aliphatic fraction in the sample and substitution 1, substitution 2 indices, and aromatic index to represent the aromatic fraction of the sample. In addition to this, the maturation degree of oil is also calculated by taking the ratio of the aliphatic index over the aromatic index. The characteristic peaks for aliphatic hydrocarbons are found at 3100–2800 cm^{-1}, 1460cm^{-1}, 1377cm^{-1} and 720 cm^{-1} and for aromatic hydrocarbons at 1600 cm^{-1} and 900–700 cm^{-1}. Notable peaks with large areas were observed at 2919 cm^{-1} and 2850 cm^{-1}, indicating a C-H stretch in alkanes representing a higher aliphatic composition. On the other hand, peaks of aromatic hydrocarbons are detected at lower levels, indicating a less aromatic fraction and hence less degradation of tar residues could have occurred. Bands within the region 870 and 730 cm^{-1} are relatively smaller (Fig 8 & 9). Hence, tarmat residues would contain less asphaltene and resins.

A similar observation has also been noted for a study conducted for crude oil using FTIR analysis (Abulkadir et al., 2016). A notable C=O peak observed at 1700 cm^{-1} for Al Zubara (Fig 10) tarmat sample indicate that the sample might have photo oxidized or degraded, with increase in polar components concentration. These peaks were not observed for tarmat samples from northern coasts (Abu Dhalouf and Al Ruwais), and this could add a piece of further information that northern coasts are still receiving fresh tar which is well indicated by earlier studies conducted in Qatar. The higher aliphatic index is observed for western coasts, whereas the higher aromatic index is observed for the eastern and northern coasts (Table 5). Results from the substitution index further indicate that weakly condensed aromatic structures characterize the western and southern coast tarmat samples (Fig 10 & 11).

Western and southern coast samples would have undergone photooxidation compared to northern and eastern samples, resulting in a decline in aromatic fraction due the conversion of aromatic to polar compounds. This could be mainly due to the oxidative cleavage of aromatic rings or with addition of oxygenated groups such as hydroxyl or carbonyl (McKenna et al., 2013). Hydroxyl group peak at 3400 cm^{-1} is not clearly detectable in tarmat samples along the coastline. However, sulfoxide peak at 1030 cm^{-1} is with higher peak areas are observed for Abu Samra (peak area = 0.2183) from the southern coast and Al Zekreet (0.0689) and Zubara (0.0871) along the western coast comparatively to the tarmat samples from northern coast such as in Abu Dhalouf (0.0072), Al Ruwais (0.0042) and Al Ghariyah (0.0011) and Fuwairit (0.0003) along the eastern coasts.

In addition to this, western samples have shown more maturation of tar (Fig 12). The major reason for these observations comparing with GC analysis is that western and southern tarmat samples are highly weathered compared to northern areas. Northern coast appears to be receiving fresh tar in limited volumes, which is obvious as the northern coast is mainly seen as Qatar's Oil hub.

5.3 Understanding the Variations in Genomic Patterns among the Bacterial Communities

Historically, the study of bacterial species and their diversity relies on environmental microbial cultivation. However, cultural studies provide little details on population structure as a result of a lack of knowledge of basic growth needs, and most bacteria cannot be cultured in the laboratory. This left much of the microbial population unexplored. Cultivation-related advances in culturally-dependent techniques as soil DNA sequence analysis rRNA 16S and nifH genes have allowed studies in the microbial diversity of the prokaryotic community to be carried out by comparing soil and other environments (Mirza et al., 2014; Hakim et al., 2018). Such experiments have

led soil microbiologists to seek more concentrated to isolate useful microbes. The researchers classify new taxa and analyze the diverse microbial flora present in soil by formulating strategies for next-generation sequencing and comprehensive metagenomic methods (Stevenson et al., 2004). These "uncultured" micro-organisms are a large and undisturbed community of diverse biological species. Uncultured bacteria are metabolically involved, but cannot proliferate in the laboratory environment in their natural habitat (Nicholas et al., 2008). The misname "unculturable" does not mean that such organisms are not ever willing to be proliferated. It means that their habitats, abiotic-biotic and ecological place in their natural environment are not properly known. Scientists are still looking for strategies for developing uncultivated bacteria. However, over 99% of soil bacteria are unculturable (Ferrari et al., 2005; Vartoukian et al., 2010).

The phylogenetically diverse organisms related to tarballs are well known. Such microbes help in biodegradation of tarballs . The structure of bacterial decaying hydrocarbon organisms is very specific and is highly dependent on the hydrocarbon composition (Bacosa et al., 2015a; Bacosa et al., 2015b). The source oil type, associated microbes and environmental conditions are considered critical for microbial tarball degradation. Tar residues become more viscous at a warmer temperature, helping microorganisms for a better degradation. However, most of the researches on hydrocarbon-degrading bacteria in Qatar is more focused on isolation from heavily polluted soils. Considering the metabolic activity of bacteria, associated microorganisms related to oil-contaminated soils could be good background information for understanding those associated with tarmats.

For this study, an unculturable technique is applied for the isolation of bacterial communities from tarmat. The tarmat samples from sixteen locations have been chosen

to identify the bacterial species using 16s rRNA metagenomic analysis. Initially, DNA has been extracted from tarmat samples using a DNA isolation kit, that is more commonly used for soil samples. A similar isolation kit has been previously used for extracting bacterial DNA from environmental matrices including tarballs (Chaudhary et al., 2019; Bacosa et al., 2016). However, a little modification regarding incubation temperature and vortex timing have been carried out for extracting DNA with maximum integrity. The DNA concentration has been assessed using the optical density method by spectrophotometer and gel electrophoresis. Absorbance at 260/280 ratios is measured using a Nanodrop spectrophotometer to produce more accurate, reliable and reproducible results. Most of the extracted DNA samples showed a high ratio of 1.7 - 1.8, except the eastern Fuwairit Sample (Fig 14). Lower concentration of DNA in the Fuwairit sample could be due to the presence of more sand content in the tarmat samples. Lower A260/A280 ratios indicate contamination by proteins and some salts or solvents. Agarose Gel Electrophoresis is further carried out to check the presence of DNA in the sample. More clear bands were not obtained since time should be increased to 40 minutes for proper migration of DNA through the gel. However, the quality control test carried out using the bioanalyzer at Macrogen Inc (South Korea) confirmed that all the samples had passed the quality control to carry out the metagenomic analysis (Table 6). The short sequence of the 16S rRNA gene was compiled using Illumina sequencer. Raw data generated after sequencing are used to have more understanding of the composition of sequences in the sample. GC and AT content percentage estimated for all samples are in the range of an average of 55%, which can be considered as a high GC content for bacterial species (Fig 16). The mole percentages of guanine + cytosine (G+C mole percentage) became the first property of DNA to taxonomical applications. For bacteria, this attribute ranges from 25 to 75%, but it is stable for a

specific individual. Close-related entities have nearly similar GC profiles and taxonomically-related groups, vary just from 3% to 5% (Tiedje et al., 1999). However, similar GC profiles do not always indicate a confirmation of relationship among bacterial communities and even a small difference is highly worth in identifying a missing relationship.

5.4 Diversity of Tarmat Associated Bacteria of Qatar Coastline

The OTUs were used to create curves for rarefaction and measure the diversity of organisms with indices including Shannon, Chao1, InvSimpson. . Rarefaction curves (Fig 19) indicated greater species diversity in northern coastal tarmat samples, such as Abu Dhalouf. The north coast tarmat samples displayed higher Shannon Index values comparatively with other coast. This also indicated north coast tarmat contained a higher diverse of bacterial communities. The Chao1 species richness indices in northern samples were also high (1917, Table 11) in contrast to for the east coast tarmat sample, such as Al Ghariyah (480), supporting the presence in the northern sample of more varied bacterial populations. It is extremely hard to categorize these bacterial communities based on the structural and physical characteristics of tarmats, as there were variations of tarmats along the coast of Qatar.

A minimum of 10 phyla from the sampled sites were retrieved. The major abundant phyla recorded was the Proteobacteria, Actinobacteria, Bacteroidetes, Proteobacteria showed about 53.95% (Abu Dhalouf) - 90.66% (Umm Tais) in the northern coastal samples, around 47% of the west and south coast and about 52.46% of the tarmat samples on the eastern coast (Figure 17). The collected tarmats were found to be dominated by proteobacteria. The tarmats collected were found to be dominated by the Proteobacteria. The phylum Proteobacteria includes heterotrophic organisms which are found in marine surface environment (Stevens et al., 2005). Most of the phyla

were not categorized at the family and genus. Major genera belonged mainly to alpha and gammaproteobacteria. Related results are recorded in during oil spill events Mexico Beaches (Kostka et al., 2011) and Norway (Brakstad et al., 2015) with higher abundance for gamma proteobacteria. Buhuring and Elivert (2005) have suggested that the coastal sands typically care dominated by phyla Proteobacteria, Plantomycetes and Bacteriodetes. This may be one of the explanations for the abundance of these genera in our samples, but bacteria can also follow a certain biodegradation route for soils and tarmats.

The Phylum Thermotogae genus *Petrotogae*, *Parvularcula* and the Deferribacter genus is exceptional in our analysis in contrast to other studies. Thermotogal enzymes are known to be active at high temperatures. Thermotogales are highly thermo-friendly and thus suitable for many applications, for example in the chemical and food industries (R. Huber et al., 2004). They can grow in hyper-saline and low-saline environments and with a maximum growth limit of 90ºC, and hence are highly capable growing in in high-temperature habitats such as deep-sea marine systems and oil field. The *Parvularcula* isolates are gram-negative, strictly aerobic and chemoheterotrophic and is considered as a marine bacterium (Cho, 2003). Deferribacteraceaa are gram-negative and use iron, manganese or nitrate to perform anaerobic respiration (Huber and Stetter, 2001). They are basically chemoorganotrophs and are linked to anaerobic respiratory processes such as nitrate reduction.

In order to obtain insight into the pathogenic potential, the bacterial taxa contained in the samples is compared with the associated literature. There were some well-known pathogenic bacterial groups in the tarmat samples including Vibrio, Rhodococcus and Acinetobacter, but their relative abundance recorded was very low. Vibrio vulnificus is one most prevalent pathogenic species. Identified by Tao et al.,

(2011) in north-central Mexico Gulf oil spill studies. The study showed V. vulnificus numbers were ten times higher in tarballs than in sand and up to 100 times higher higher than in seawater. This is highly noteworthy because it is very rare to have higher counts of V. Vulnificus in environmental samples (DePaola et al. 1994). However, Vibrio was reported in very low relative abundance in all of the tarmat samples in the present study.

5.5 Hydrocarbon degraders and pathogenic groups among the tarmat associated bacteria of Qatar coastline

Hydrocarbon-degrading microorganisms are widespread in the oceans and the coastal environment and are widely studies (Head etal., 2006; Yakimovetal. ,2007) and the final fate of much of the oil hydrocarbons that join such environemnt is indigenous biodegradation (Leahy and Colwell,1990; Prince,2010; Atlas and Hazen,2011). Different marine microorganisms have evolved with similar complexity of metabolic pathways to use hydrocarbons as a rich carbon and energy supply in reaction to the natural nature of petroleum compounds. While biodegradation has shown progress in remediating naturally occurring oil pollution linked to many marine shoreline spills (Head et al., 2006; Prince, 2010, Atlas and Hazen, 2011), more need to be understood regarding environmental controls on hydrocarbon depletion in marine environment.

Only few genera such as *Marinobacter* and *Alkanivorax* were classified as likely by the overall bacterial diversity in the tarmat samples as a hydrocarbon degrading genera, primarily of the phyla proteobacteria. Alphaproteobacteria may be correlated with degradation of LMW PAHs, whereas degradation of HMW PAHs may be correlated with gammaproteobacteria such as *Marinobacter. Marinobacter* is renowned for its capacity to denitrify and flexibly using a wide variety of substrates, including alkanes and PAHs (Head et al., 2006; Berlendis et al., 2010). Previous findings shows that *Marinobacter* are even more metabolically efficient in mitigating oil pollution.

Degardation of large n-alkanes and branched alkanes, like pristane and phytane, are sometimes correlated with *Alkanivorax* genera (Hara et al. 2003; Harayama et al . 2004). The major family of hydrocarbon-degrading capabilitiy in the studied tarmat samples include Rhodobacteriales, Oceanospirillales, Alteromondales and Sphingomonodales. Rhodobecteriales is known to have occurred in contaminated water and beach sands after the DHW spill, particularly in degradation of aromatic hydrocarbons such as naphthalene and phenanthrene (Kostka et al., 2011; Goutierrez et al., 2013; Dubinskyet al., 2013;). When members of oceanospirillas were abundant, the highest proportion of n-alkanes and cyclo-alkanes were found in marine waters during DWH spill (Hazen et al., 2010; Mason et Al., 2012; Dubinsky et al., 2013).

The existence of *Alcanivorax, Psychrobacter and Pseudoalteromonas* dominates the analysis performed by Bacosa et al . , (2016) on tarballs from Galveston and Mustand Island, Texas. The study found that Galveston Island tarballs are dominated by *Alcanivorax and Psychrobacters* showed a 21% depletion of C9-C17 alkanes and 55% degradation of PAHS. PAH reducing *Psuedoalteromonas,* on the other side, controlled tarballs from Mustand Island and had a 24% alkane and 63% PAH depletion. Here *Psuedoalteromonas* domination has been correlated with older tarballs. In the tarmat samples of the present study no such significant bacterial community shift was observed , for example from an alkane degrading community to a degrading PAH community. The genera *Thermodesulfobacterium*, for example, was seen at some sites, but more frequently at Port Island (Northern Caost) and Al Ghariyah (Eastern Coast). Genera *Salimonas* was only found in tarmat samples from the north coast of Umm Tais. Genera such as *Alcanivorax, Alkalimincola, Defluvicoccus, Petrotoga, KCM B-112 and Marinobacter* exhibited variations in their lower to higher abundance along the coastline of Qatar and did not display very interesting bacterial

transition. This would lead us to understand the importance of identifying these bacterial species' degradation capabilities. Throughout this analysis a non-cultural technique was used to investigate bacterial populations, while a cultivable technique can be used to consider its degradation capabilities and to evaluate the chemical composition using the GC-MS model. Few experiments have followed a reliable approach. In his research, Shinde et al . (2017) isolated 49 tarball-related bacteria from Betul Beach in Goa , India. The gene-sequence analyses identified phylogenetically diverse 20 bacterial genera belonging to the phyla Proteobacteria (14), Actinobacteria (3), Firmicutes (2) and Bacteroidetes (1). In his recent study, Shinde et al. (2020) have screened 38 tarball-related bacteria for n-alkane and PAH crude oil degradation. *Alcanivorax, Marinobacter and Pseudomonas* were major genera considered for the preparation of bacterial consortiums. Consortium of bacteria like Pseudomonas sp. Betul 14, Pseudomonas sp. Betul 14 Betul-M and Sp. Betul-M. Betul-O demonstrated impressive tarball degradation capabilities within 45 days, with 97.78% and 61.98% respectively, of n-alkanes and PAH degradation. A related strategy could be regarded for the tarmats samples in our study as *Alcanivorax and Marinobacter* were one of the dominant hydrocarbon degradation capabilities correlated with abundant genera observed. Moreover, it may also be beneficial to screen live Cultures from the genera *Petrotoga, Parvularcula, Deferribacteres, Salinimonas* in evaluating their capacity for the degradation of hydrocarbons on tarmates along the coast of Qatar as these were unique genera compared to what observed in previous studies. This has been reported that previously independent bacterial populations had less potential to decay than bacterial consortia. However, a positive finding may be obtained from a bacterial consortium containing *Alkalimnicola and Alcanivorax, Marinobacter* and Alcanivorax from phylum proteobacteria. Shinde et al. (2020) have also reported the

depletion of isoprenoid alkanes by the bacterial consortia *Marinobacter and Alkanivorax*. C17 / Pristane and C18 / Phytane ratios are commonly used as markers to identify biodegradation effects because n-alkans are likely to be biodegraded relative to isoprenoids (Wang et al.1998, 1999). *Alkanivorax* is known to be an important alkane degrader for its hydrocarbonoclastic capacity and is a prinicipal alkane degrader. *Glacieola* from phylum proteobacteria in lower abundance has also been reported in t his study. While oil depletion is not well established for this species, studies indicate that it is prevalent in oilcontaminated cores of mined Arctic ice (Brakstad et al., 2008). In research conducted by Chronopoulou et al . (2015), however, on the Glaciecola strain obtained from tetradecane, the Antarctic enrichment did not directly contribute to the degradation of hydrocarbons, but rather demonstrated their capacity to produce or expand in the presence of alkanes. Pseudomonas bacteria are considered to be competent hydrocarbon degraders in studies previously performed in Qatar. Bacosa et al . (2013) identified Pseudomonas as one of the most prevalent genera of heavy oils, the main cause of aliphatic and aromatic compound degradation. Owing to its versatility and capacity to biodegradate hydrocarbons, Pseudomonas aeruginosa is known to be a effective bioremediation organism for hydrocarbon-contaminated habitats. Hydrocarbon degrading genera reported from various studied conducted in Qatar is reported in previous section, chapter 2. Similar communities were not found in our present tarmat study. Considering the harsh and unique climate conditions in Qatar, one could always assume of similar communities to be found in tarmats as well, especially of Pseudomonas. Major reason could be a different biodegradation pathways could be exist for both tarmats and soil. Unfortunately, no study has been documented explaining the biodegradation pathways tarmat or tarball associated bacterial communities. Hence, understanding their metabolic and catabolic pathways and their degradative genes

would further help us to demonstrate the differences between the bacterial communities observed in tarmats and coastal sediments along the Qatar coastline.

CHAPTER 6: CONCLUSIONS

Petroleum input from marine transport and accidental oil spills have been drastically increased since past few decades causing catastrophic accidents for both marine and coastal ecosystem including severe economic and environmental damages. The tar deposition on the Qatari beaches highly varies, depending upon location. More understanding of tar deposition could be acquired while incorporating both meteorological and hydrological conditions. This is as most important for better remediation and mitigation measures along with understanding the bacterial communities. Aboul Dahab (1994) in his earlier study, stated that a clean-up activity had been carried out on the western side of Al Ruwais by the Environment Protection Committee Qatar. He further indicated that even though the result has been obvious, most of the intertidal zones were still coated by oil along with a huge pile of debris with additional damage to the rocky substrate.

The major objective of this study is to link the chemical composition of tarmats to their associated bacterial communities to know more about PAH and alkane degraders. The concentrations of PAHs and TPH have been determined using GC along with understanding a qualitative degradation of tarmats along the Qatar coastline using ATR – FTIR technique. The results from the study indicated that Infrared spectroscopy is indeed a viable tool for characterizing tarmat and understanding their chemical composition. Bacterial communities have been identified via high throughput sequencing by metagenomic and bioinformatic analyses. Our study confirmed the presence of oil contamination along the Qatari coastline, heavily contaminated along the north and northeastern coastline. Highly weathered or matured tarmats have been observed along the western side, indicating higher aliphatic content than aromatic content. Higher aromatic content observed along the northern coast confirmed that these regions are always exposed to fresh oil coating through oil spillages and other

petroleum industrial activities.

Investigating the bacterial communities through 16s sequencing and bioinformatic approach have led to understanding of diverse bacterial communities contained in tarmats along the Qatar coastline. Ten phyla were recorded in which Proteobacteria, Thermotoga, Deferribacteres, Cyanobacteria, Bacteriodetes and Actinobacteria were the dominant. However, most of these are unclassified at the genus level and were classified more into gammaproteobacterial and alphaproteobacteria. Marinobacter and Alkanivorax were the major dominant hydrocarbon degrader and no prominent pathogenic bacterial community were identified in the study. Absence of estimating the diagnostic ratios and understanding in more depth about the chemical composition rather than determining the concentration could have improved the purpose of the study in a more better way to link the bacterial communities in tarmats with their hydrocarbon composition. Some of the tarballs which are found more soft , sticky and goey could be in their early stage of weathering process and hence enumeration of the density of the bacteria would be highly difficult. Advances in next-generation sequencing technology and the usage of stable isotope tracers may have significantly improved our capacity to challenge hydrocarbon-degrading microorganisms' phylogenetic and functional complexity in the region. Hence, a great need lies there to understand the complex process of metabolic pathways of these indengenous hydrocarbon organism. This is quite important because it is not always possible to treat biologically and bioremediate hypersaline environments with conventional microorganisms due to their inefficiency. It has been proven that halophilic communities are more efficient in bioremediation and degradation of organic pollutants. Hence, understanding the PAH catabolic property of the halophilic dominant groups observed in our study such as Alphaproteobacteria, Gammaproteobacteria,

Actinomycetes and Firmicutes would be quite interesting and useful for further researches related to bacterial communities associated with tarnats along the Qatar coastline.

In the present study, we adapted a non culturable to understand the bacterial communities which indeed provided us with a very promising result. It is quite known that not all microorganisms are culturable under laborartory conditions. However, the physical structure of tarmats and tarballs makes them quite difficult for a culturable technique and is quite uncertain. In than casem several additional treatment of tarballs including biostimulation should be considered. Metagenomic along with bioinformatic would help to understand the diversity of bacterial communities, however such is not a favoured approach for bioremediation. There are only very limited studies conducted on tarball associated bacterial communities based on both culturable and independent procedures. In Qatar, no such research is conducted but there is excellent literature available on bacterial communities related to oil polluted soil. This would provide a great background information in studies dealing with tarmats to understand how they are well distinguished .

Major categorization of remediation strategies falls into three important categories: physical, chemical and biological. Contamination levels project feasibility and service quality, including the technical support, determine the choice of any clean-up technique, and it is proven that some bioremediation techniques are 95% effective in the degradation of oils. However, through the natural remediation process, it has been documented after the Gulf war that vast stretches of algae have evolved from oil reserves by growing organotrophic organisms and using crude oil as a source of carbon and energy. This natural degradation is attributed to areas with higher temperatures and higher sunlight and rapid bacterial growth. However, this natural process is as well dependent on meteorological conditions and environmental sensitivity. The

bioremediation process that uses the indigenous hydrocarbon-degrading organisms of contaminated soils is known for many years. Even though some of the techniques have gone through a successful implementation, this field requires a lot of research and interest (Das and Chandran, 2011). Applying the C / N / P ratios is a common method in bioremediation. Because of low hydrocarbon bioavailability and different elementary cell composition of the generated biomass, the C / N / P ratios supporting good hydrocarbon degradation are lower than the specific bacterial requirements. This is because rather than integrating with microbial biomass, carbon from most of the pollutants is mineralized into carbon dioxide and water for energy production. Rhodococcus and Pseudomonas strains, when cultured on PAH, were characterized by high mineralization yields at certain C/N/P ratios along with higher cell production and larger accumulation of metabolites (Leys et al., 2005). However, the lack of information regarding the indigenous bacteria that survive in harsh and conditions reflects a poor chance of enhancing their growth for bioremediation approaches. Reducing the environmental impact of oil spills and optimizing the environmental benefits of biodegradation, the metabolic ability of hydrocarbon-degrading bacteria must be identified and the factors preventing microbial-catalyzed biodegradation in situ discussed.

CHAPTER 7: REFERENCES

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APPENDIX

1. ATR – FTIR spectra of tarmat samples along the Qatar coastline

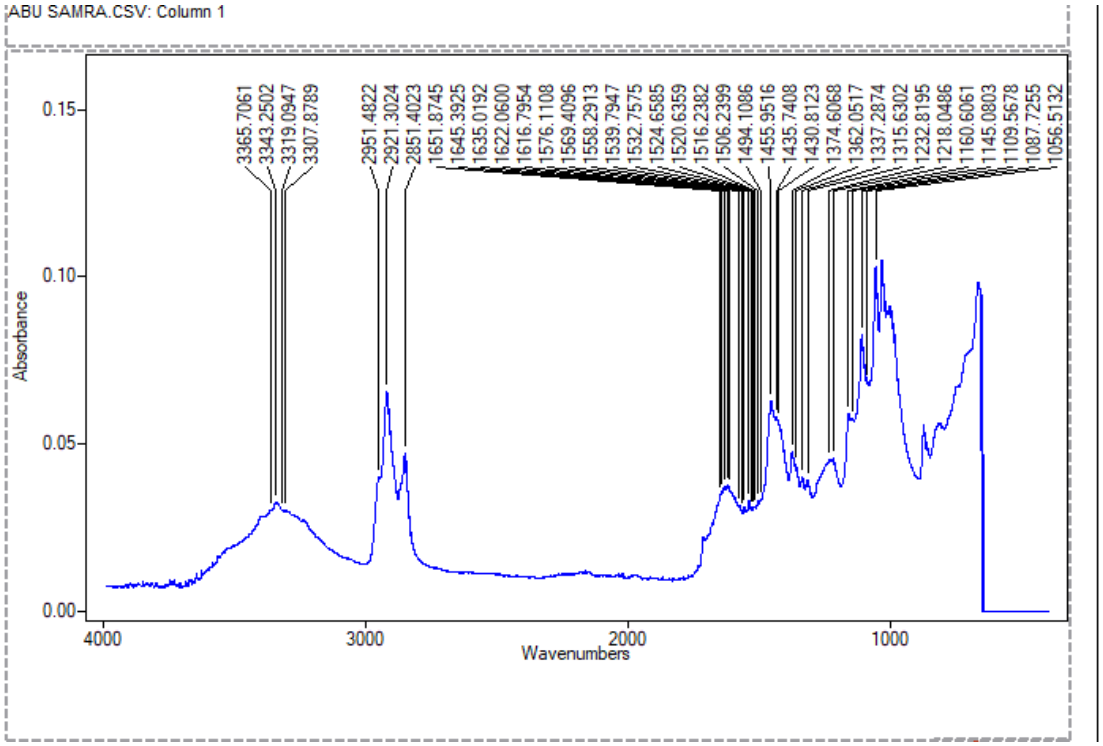

Figure 1.1 Abu Samra

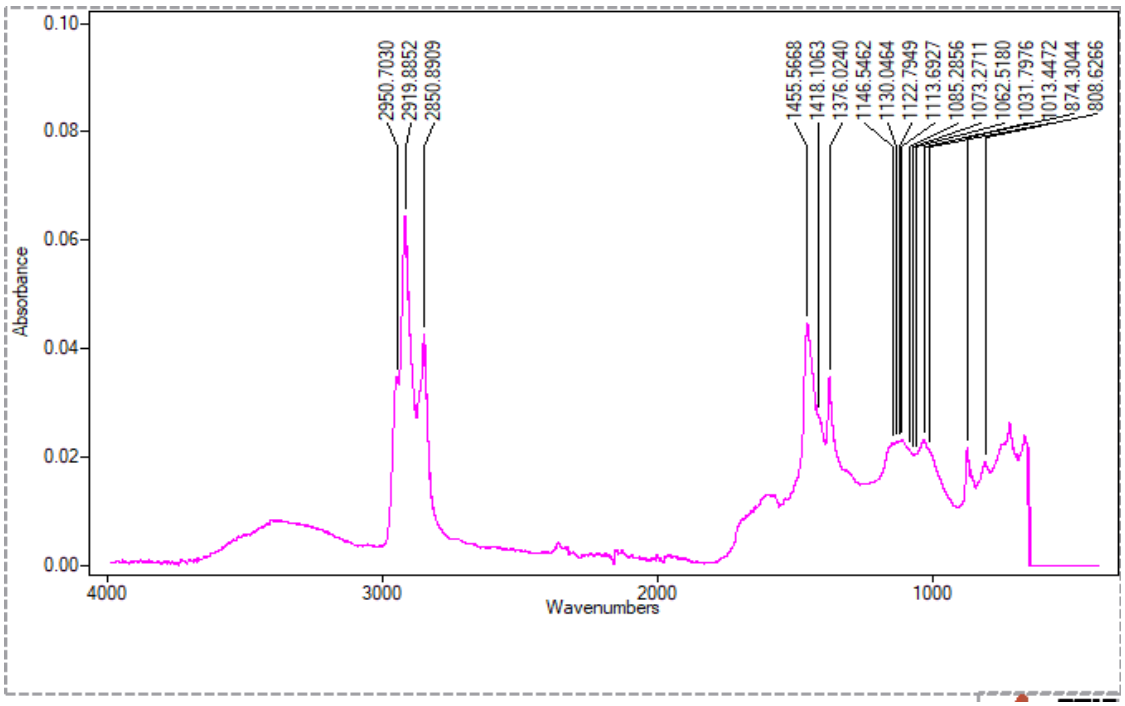

Fig 1.2: Al Arish

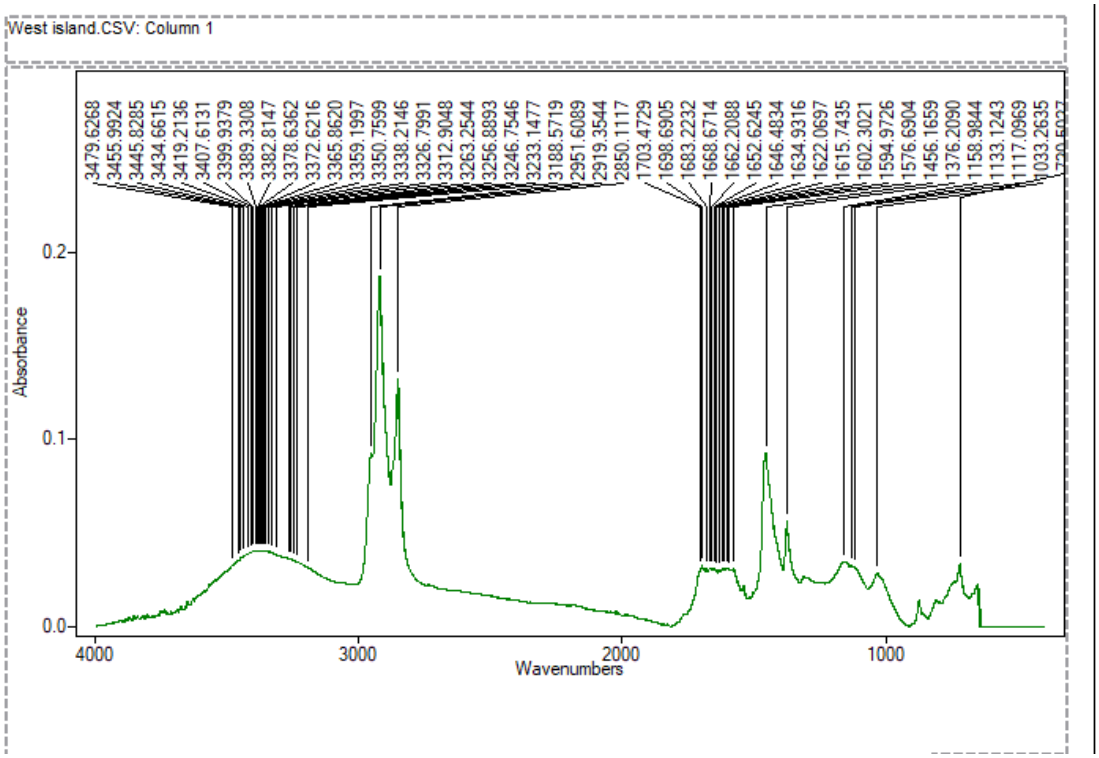

Fig 1.3: Zekreet West Island

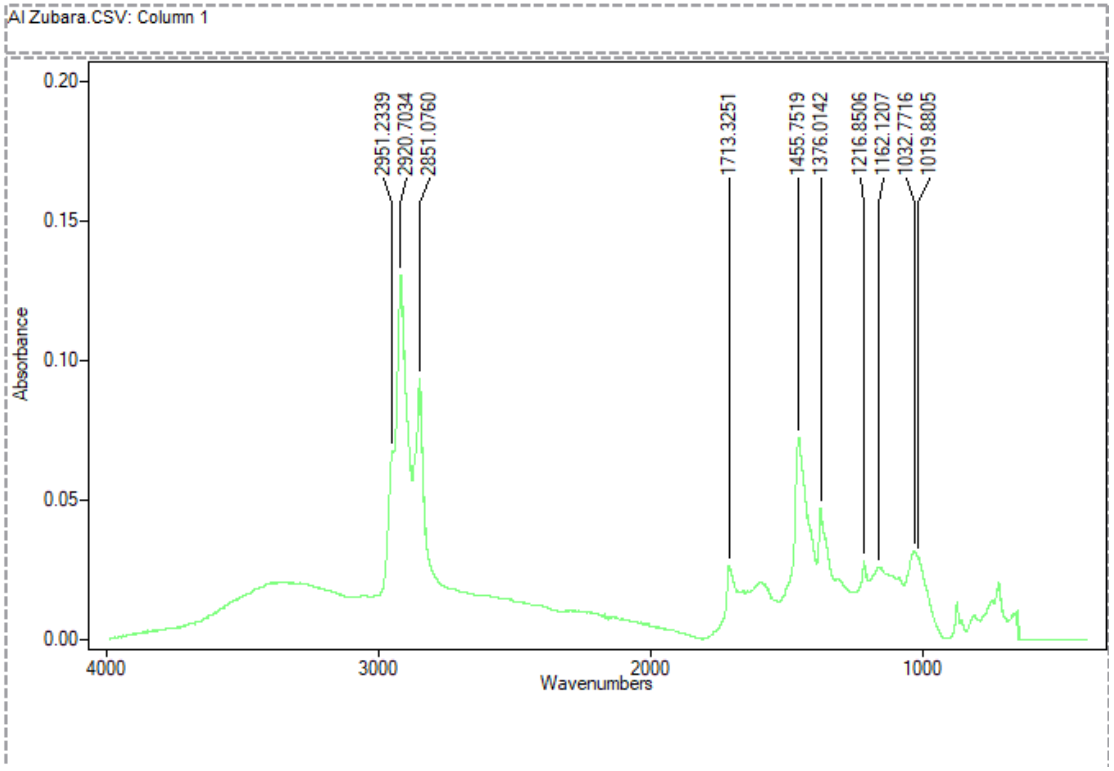

Fig 1.4: Zubara

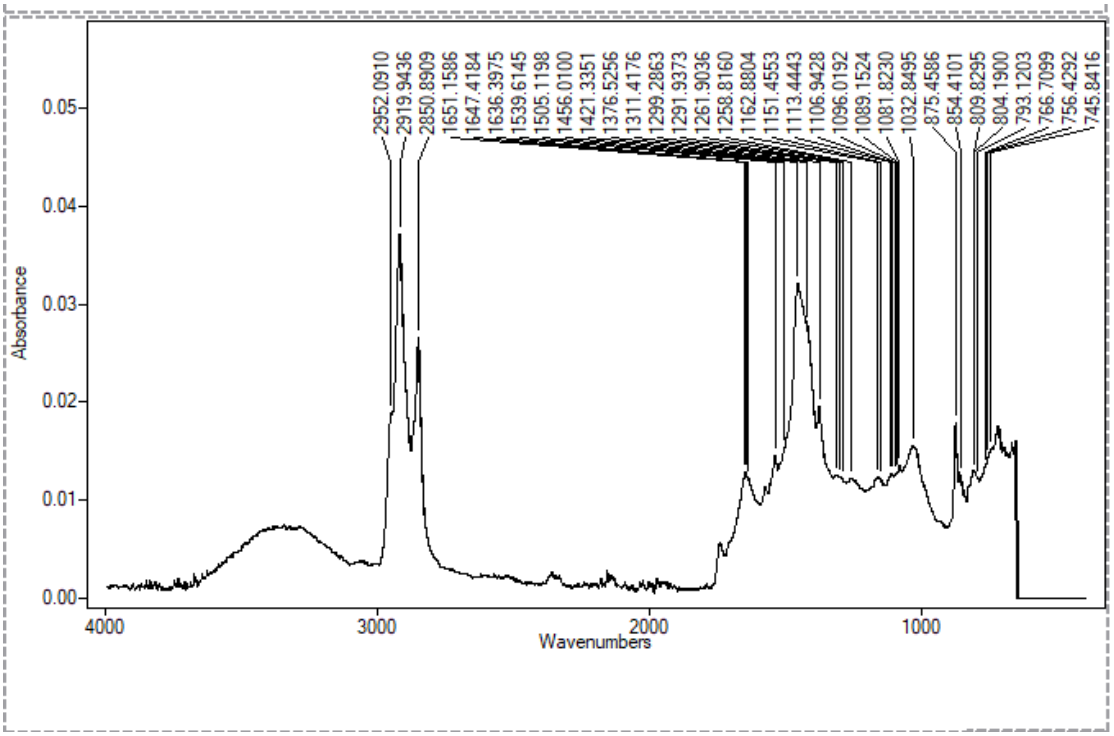

Fig 1.5: Al Ghariyah

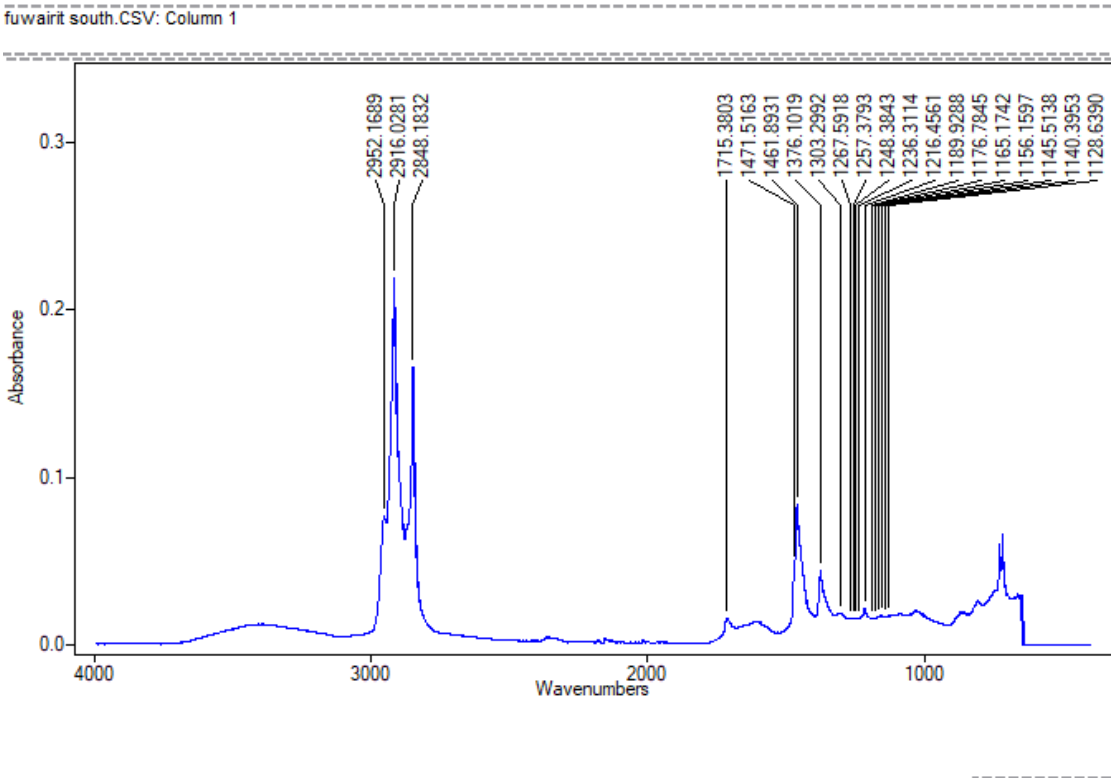

Fig 1.6: Fuwairit

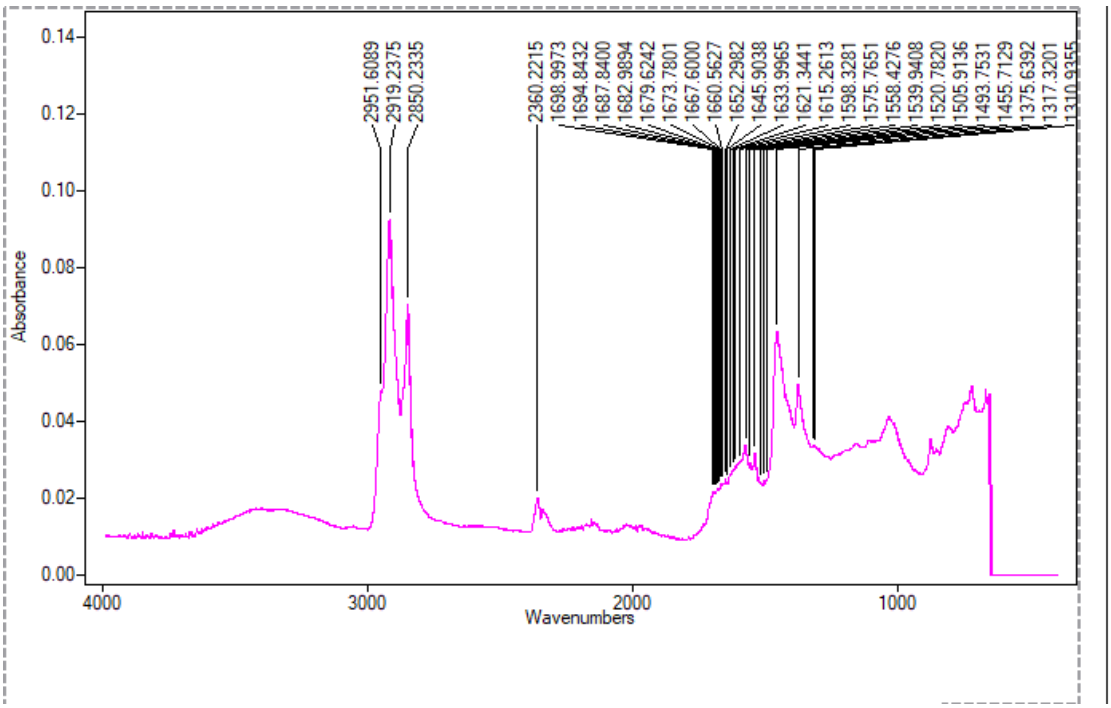

Fig 1.7: Lejnail

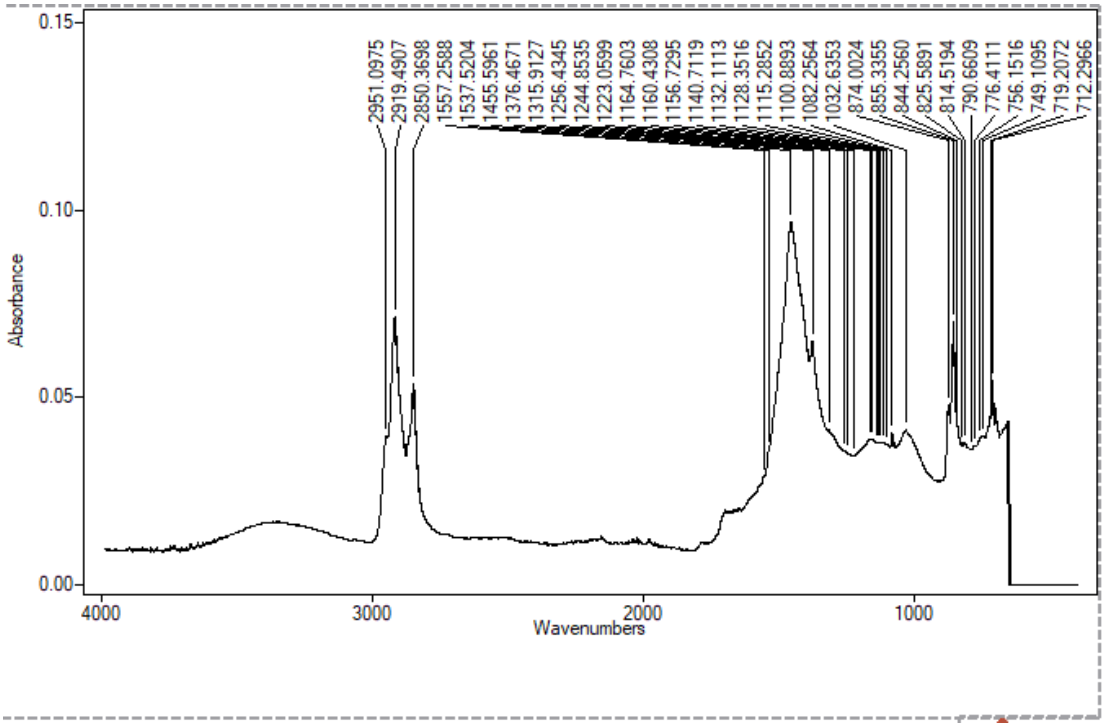

Fig 1.8: Port Unknown Island

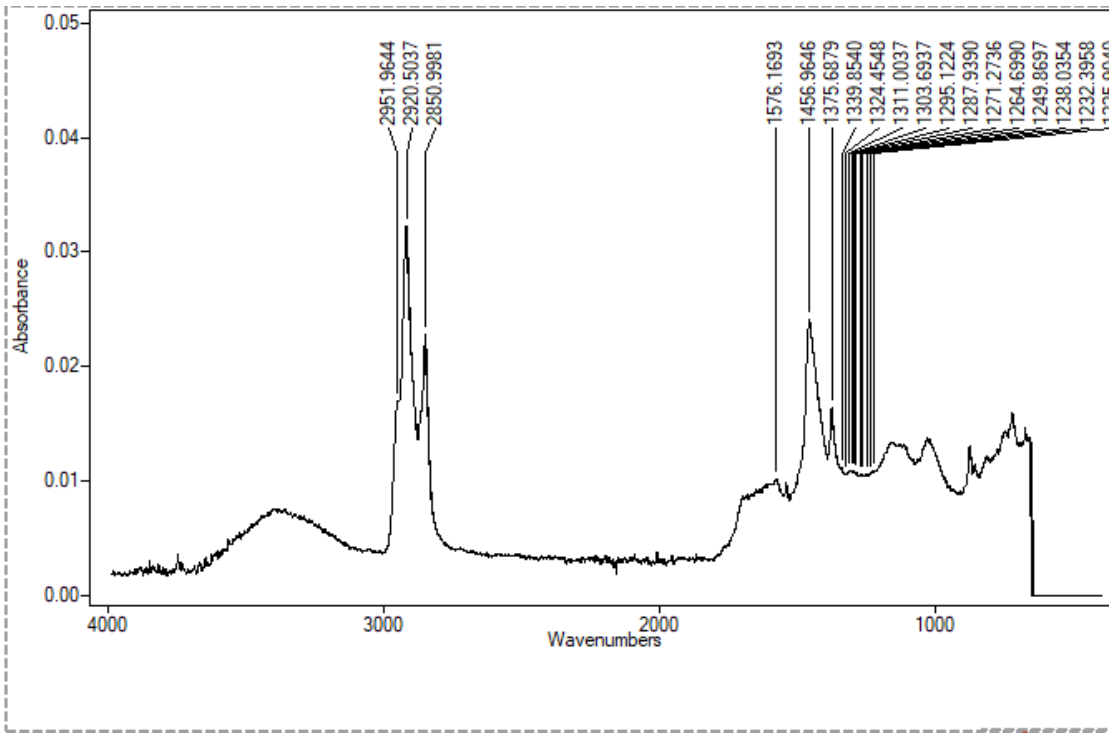

Fig 1.9 : Ras Rakan

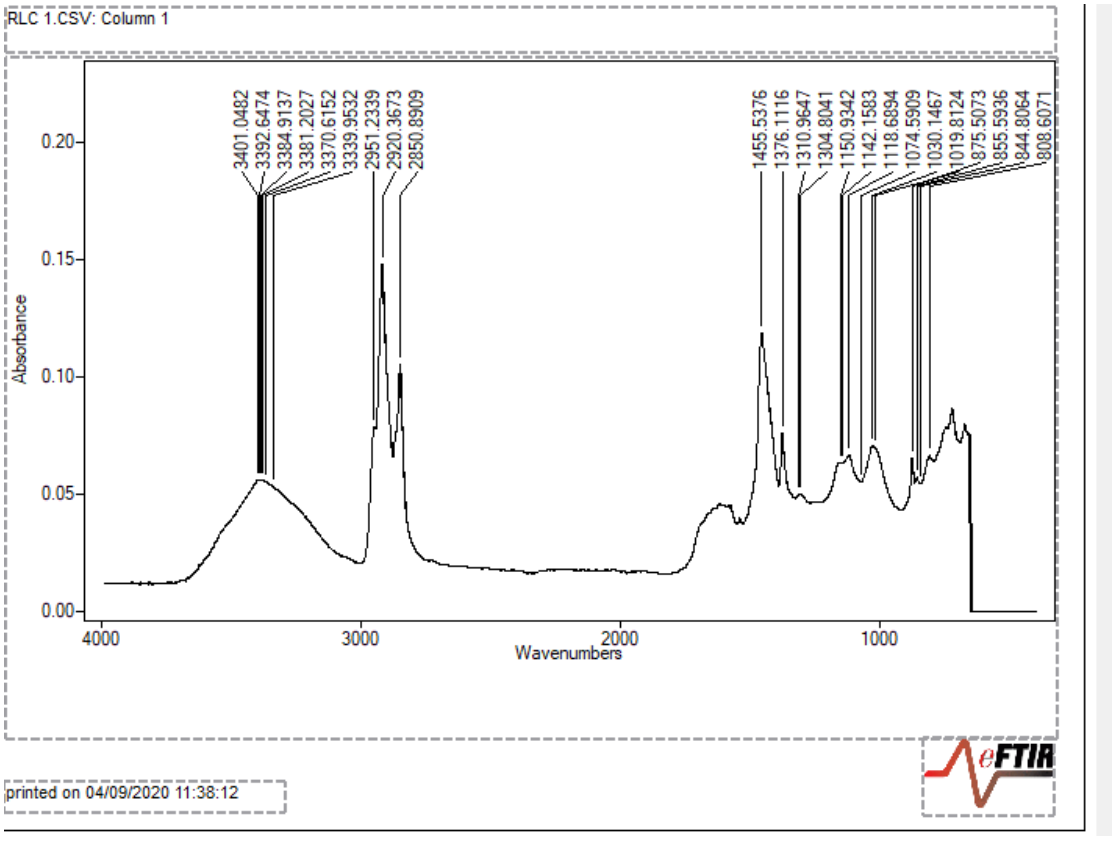

Fig 1.10: Ras Laffan

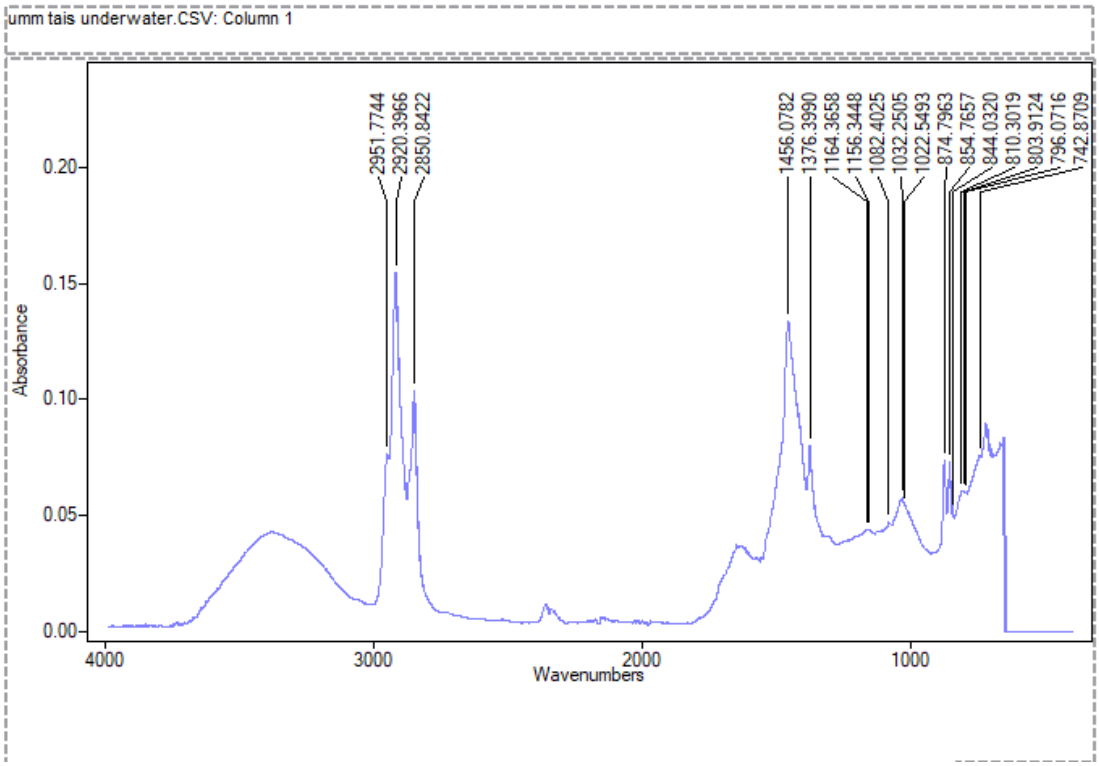

Fig 1.11: Umm Tais

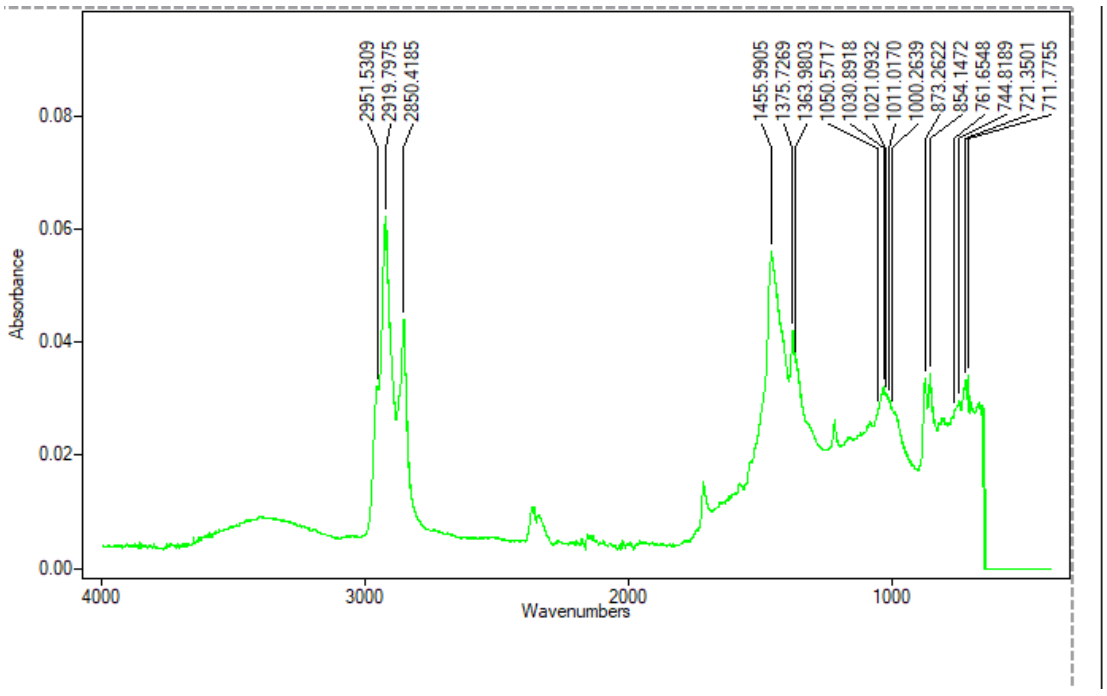

Fig 1.12: Abu Dhalouf

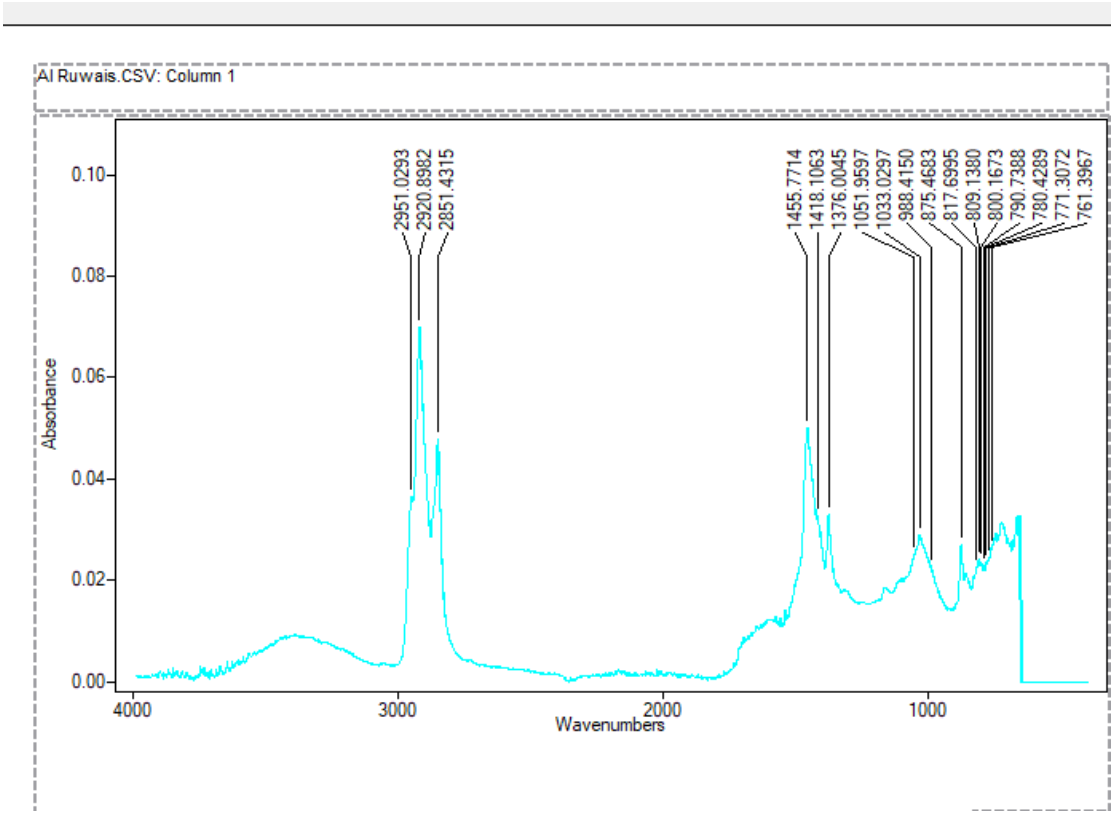

Fig 1.13: Al Ruwais

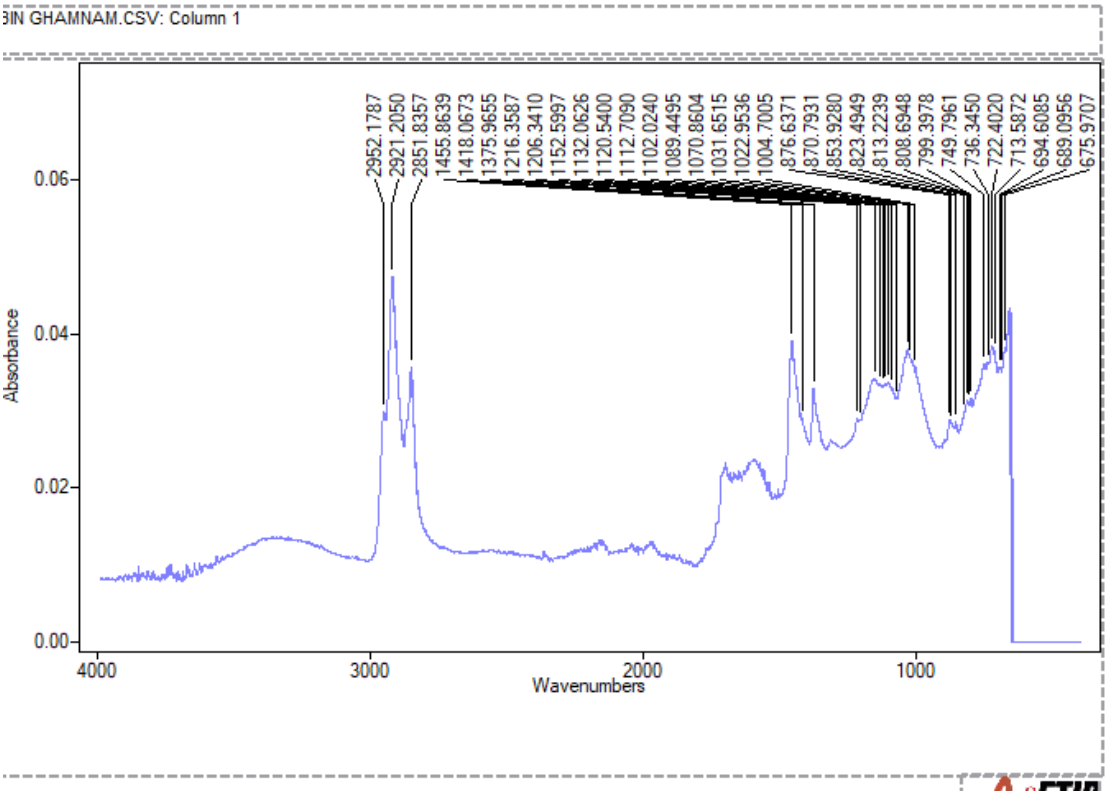

Fig 1.14: Bin Ghamnam