AMERICAN UNIVERSITY OF BEIRUT

ASSESSMENT OF THE BIOREMEDIATION OF CRUDE OIL SPILLS ON THE LEBANESE SHORELINE

by
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A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Engineering
to the Department of Civil and Environmental Engineering
of the Maroun Semaan Faculty of Engineering and Architecture
at the American University of Beirut

Beirut, Lebanon
July 2018

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ACKNOWLEDGMENTS

This research was funded by the Munib R. and Angela Masri Institute of Energy and Natural Resources. The crude oil was supplied by the Jordan Petroleum Refinery.

AN ABSTRACT OF THE THESIS OF

Khaled Jihad Sakaya for Master of Engineering
Major: Environmental and Water Resources Engineering

Title: Assessment of the Bioremediation of Crude Oil Spills on the Lebanese Shoreline

With the planned oil and gas exploration activities off the coast of Lebanon, the risk of shoreline contamination with crude oil spills has become a major concern. This study aims at assessing the crude oil bioremediation potential of the Lebanese shoreline and the efficiency of bioremediation enhancement by biostimulation, during the dry and the wet seasons.

Laboratory scale biodegradation experiments were conducted using crude oil-spiked sediments and seawater over a 42-day period. It was postulated that, since untreated raw sewage is discharged continuously along the shoreline of Lebanon, nitrogen and phosphorous nutrients may be found in the seawater at high enough concentrations to stimulate microbial activity without the need for additional nutrients enhancement. The experiments were conducted during the wet (18°C) and dry (28°C) seasons to account for temperature and nutrients variability during these periods. The biodegradability of oil constituents – namely alkanes and polycyclic aromatic hydrocarbons (PAHs), was monitored and quantified periodically using gas chromatography-mass spectrometry (GC-MS).

Little to no enhancement to the overall biodegradation rates of alkanes and PAHs was observed under the biostimulation treatment in sediments at 18°C and 28°C. Significant enhancement in the biodegradation rates however was observed in seawater at both temperatures. Under both natural attenuation and biostimulation treatments, the increase in temperature increased the oil biodegradation rates in the sediment and seawater microcosms. The results obtained from this study would guide policy makers and spill responders in developing sound environmental planning for the bioremediation of potential crude oil spills on the Lebanese shoreline.

Keywords:
Crude oil spills, bioremediation, background nutrient levels, biostimulation, marine sediments, seawater, polycyclic aromatic hydrocarbons, alkanes.

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CHAPTER I
INTRODUCTION

In 2010, the United States Geological Survey department (USGS) conducted a geological study on the Levant Basin Province, during which 1.7 billion barrels of recoverable crude oil and 122 trillion ft^3 of recoverable natural gas were estimated contained within the area, which includes Lebanon's 17,901 Km^2 Exclusive Economic Zone (EEZ) (Schenk et al., 2010). In the outcome of this study, massive oil and gas exploration activities were planned off the shores of Lebanon, raising paramount concerns on the safety of its marine environment. Risks of major crude oil spills are magnified with the increased oil-related activities, posing substantial threats on Lebanon's marine ecosystems, and ultimately on its public health (Prince, 2010).

The Lebanese Petroleum Administration (LPA) divides the planned oil-related activities over 10 blocks off the shores of Lebanon, with blocks 4 and 9 already licensed for exploration and production (Lebanese Petroleum Administration, 2017). Figure 1 locates the 10 off-shore exploration blocks, highlighting the currently open blocks for bidding during the first licensing round. In 2012, a study under the supervision of the Lebanese Ministry of Energy and Power simulated the behavior of potential oil spills in several locations within these exploration blocks, and under different scenarios. The predicted behavior of the oil slick exposes the entire shoreline to oil pollution threats in the event of a crude oil spill (Ministry of Energy and Water, 2012).

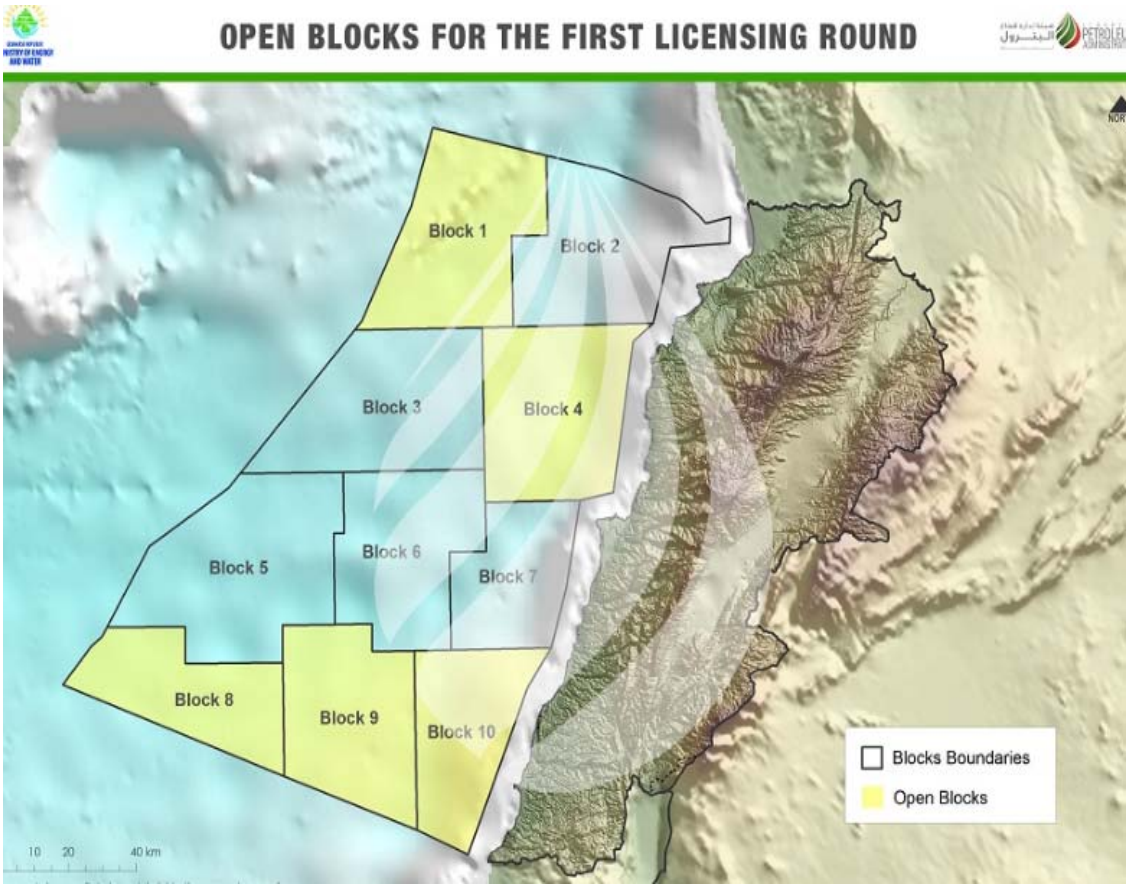

Figure 1. Open oil exploration blocks for the first licensing round (LPA, 2017).

Petroleum hydrocarbon constituents such as aliphatic and aromatic hydrocarbons and heavy metals present substantial toxic effects on the environment. These contaminants, particularly high molecular weight polycyclic aromatic hydrocarbons (PAHs), have mutagenic and carcinogenic impacts on living organisms, and are consequently classified as "compounds with significant human health risk" (Dabrowska et al., 2008). Therefore, these risks should be controlled with sound environmental planning for the sake of the environment and human beings. While Lebanon still lacks sufficient data to develop a proper oil spill response plan, urgent guidelines for policy makers and spill responders need

to be developed.

Conventional physical and chemical remedial techniques such as booming, skimming, dispersants, solidifiers, etc… (Venosa and Zhu, 2003) are usually the fisrt resort in the event of an oil spill for their rapid clean-up capability (Nikolopoulou and Kalogerakis, 2009; Röling et al., 2002). However, these conventional techniques are extremely expensive, sometimes toxic, intrusive and disruptive to the marine environment, and only partially successful in eliminating oil pollution (Nikolopoulou and Kalogerakis, 2010; Nikolopoulou and Kalogerakis, 2009; Röling et al., 2002; Atlas, 1995). Nevertheless, natural degradative processes mediated by indigenous hydrocarbon degraders can assist in removing residual petroleum hydrocarbons (Röling et al., 2002). Petroleum hydrocarbon-degrading microorganisms are ubiquitous in marine environments as a result of natural plant oil synthesis and natural oil seeps, making intrinsic biodegradation the ultimate fate of most hydrocarbons in these environments, given sufficient time (Ron and Rosenberg, 2014; Nikolopoulou and Kalogerakis, 2009; Röling et al., 2002; Atlas, 1995). However, the intrinsic biodegradation rates are slow in marine environments to effectively remediate major oil spills that could heavily contaminate marine shorelines and water columns, and toxically affect marine ecosystems, which justifies resorting first to physical and chemical removal processes (Ron and Rosenberg, 2014; Atlas, 1995). The low concentrations of utilizable nitrogen (ammonium, nitrate, and organic nitrogen) and phosphorous necessariy for bacterial growth and biodegradation processes are often the primary biodegradation rate-limiting factors in marine environments due to their high water solubility which reduces their bioavailability in open systems (Ron and Rosenberg, 2014; Atlas, 1995). Improving environmental conditions to get optimal values of utilizable nutrient levels could

achieve optimal microbial degradation rates (Tyagi et al., 2011).

Since the 1970's, bioremediation through nutrient addition (biostimulation) has proven environmental applicability, versatility, and efficacy in mitigating oil spills in marine environments as compared to physical and chemical remediation techniques (Nikolopoulou and Kalogerakis, 2010; Xu and Lu, 2010; Atlas and Bragg, 2009; Venosa and Holder, 2007). Field tests exhibited a strong positive correlation between the biodegradation rate of oil and the nitrogen to oil ratio (Atlas and Bragg, 2009). The addition of appropriate amounts of utilizable nitrogen and phosphorous in the contaminated sites stimulates bacterial growth and ensures optimal oil biodegradation rates (Tyagi et al., 2011; Röling et al., 2002; Atlas, 1995). Excessive addition of nutrients, however, does not further enhance biodegradation rates, and could damage the marine environment by causing eutrophication (Tyagi et al., 2011; Nikolopoulou and Kalogerakis, 2009; Röling et al., 2002). On average, nitrogen levels required for optimal oil biodegradation rates range between 2 and 10 mg-N/L (Tate et al., 2012; Venosa et al., 2010). In the event of the famous Exxon Valdez oil spill (EVOS), where 41.6 million liters of oil were spilled, bioremediation with nutrient addition (namely nitrogen and phosphorous) induced a 5 fold oil biodegradation rate-enhancement when applied to the shores of Prince William Sound over the summers of 1989-92, and proved safety and efficacy in removing 25% of the initial oil mass, with significant degradation of most types of hydrocarbons (Atlas and Bragg, 2009; Bragg et at., 1994). Biostimulation also demonstrated enhanced biodegradation rates of the lingering oil present in subsurface sediments in Prince William Sound 19 years after the EVOS (Venosa et al., 2010). On the other hand, a study on the bioremediation of an experimental oil spill conducted on the shoreline of Delaware Bay in

1994 concluded that biostimulation did not substantially enhance the biodegradation rates of hydrocarbons where background nutrient levels were sufficiently high to support high intrinsic biodegradation rates (Venosa et al., 1996). Another experimental oil spill conducted in a coastal salt marsh of Louisiana demonstrated a lack of biodegradation rate stimulation of branched and unbranched alkanes after nitrogen addition. This was attributed to low oxygen availability which acted as the rate-limiting factor (Tate et al., 2012). The differences between the results of the conducted studies could go back to the differences in the geology of the contaminated marine environments, the properties of the affected ecosystems, as well as the quantity and quality of the released hydrocarbon mixture (Röling et al., 2002; Atlas, 1995). This also sets background nutrient levels as a crucial factor affecting the biodegradability of crude oil, among other factors including dissolved oxygen concentrations, moisture content (in soils and sediments), temperature, and the size and diversity of the oil-degrading microbial consortium present at the water-oil interface. Optimal parameters should be ensured for an efficient natural biodegradation processes (Pi et al, 2015; Atlas and Hazen, 2011; Tyagi et al., 2011;Venosa et al., 2010).

The continuous discharge of nutrient-rich raw sewage into the Mediterranean sea along the shoreline of Lebanon (Rust and Linden, 2008), as well as the anticipated seasonal variation in background nutrient levels and temperatures between the dry and the wet seasons, make Lebanon an interesting case to assess the crude oil bioremediation potential of its shoreline, in light of the planned offshore oil and gas extraction. In October 2006, O. Linden and M. Russ (2008) conducted a field study on the distribution of petroleum hydrocarbons and PAHs in the coastal ecosystem after the major oil spill in Lebanon during the war in July 2006. It has been reported that petroleum hydrocarbon levels 3 months after

the spill were equivalent to the background concentrations often found in coastal sediments due to anthropogenic sources (Rust and Linden, 2008). This suggests a high intrisinc oil biodegradation activity in the contaminated area that could be attributed to high levels of nitrogen and phosphorous available to the hydrocarbon-degrading microbial communities in Lebanon's marine environment.

Therefore, the aim of the present work is to assess the effect of biostimulation on the biodegradation rates of petroleum hydrocarbons in oil-contaminated beach sediments and seawater along the Lebanese shoreline. Beach sediments and seawater were characterized for their oil-degrading microbial community concentrations and background nutrients levels – namely nitrogen and phosphorous. The biodegradation experiments were conducted under natural attenuation and nutrient-amended conditions (N and P) to enhance the microbial activity. The experiments were carried out during the wet season at 18°C and during the dry season at 28°C, to account for the seasonal variation in temperature and nutrient levels. The biodegradation of oil constituents, namely alkanes and PAHs, was monitored and quantified in sacrificed microcosms throughout the experiments using gas chromatography – mass spectrometry (GC-MS).

CHAPTER II

EXPERIMENTAL SECTION

A. Samples Collection

Eleven sites along the coast of Lebanon were selected to cover its beach sediments and seawater characterization during both the wet and the dry seasons (figure 2). The designated sites, except for Sarafand, consist of sandy beaches and were distant from nutrient-rich effluent sources for a more representative characterization. Samples from the shores of Sarafand and Naqoura were discarded form the second shoreline characterization (wet season), due to the rocky nature of the shores of Sarafand, and the restricted access to Naqoura at the time when the sample collection was scheduled.

Shoreline surface sediments were manually collected at water depths ranging from 30 cm to 50 cm, while seawater samples were taken from the top 30 cm of the surface water. Sampling was done in triplicates at each location, and the samples were transported on ice to the laboratory where they were preserved at 4°C until used for chemical analysis 24 hours post-collection. The bacteriological analysis for quantifying the background concentrations of alkane and PAH-degrading bacteria was conducted immediately on the day of sampling.

Figure 2. The selected sampling sites for the shoreline characterization.

B. Sediments and Seawater Characterization

For the chemical analysis, sediments and seawater samples were assessed for their nitrates-N, nitrites-N, ammonia-N, total kjeldahl nitrogen, total nitrogen, phosphate-P, and total phosphorous contents by spectrophotometry using standard Hach methods. Sediment samples were sieved with sieve No. 10 (2 mm pore size), and 30 g-aliquots were mixed with 100 mL distilled water at 300 rpm for 1 hour to extract the chemical compounds. The chemical analysis was performed on the extracts. No sample processing was done with respect to seawater samples.

For the bacteriological analysis, the samples were processed for the most probable number (MPN) analysis for alkane and PAH-degrading bacteria. For seawater, the samples underwent serial 10-fold dilutions in 96-well microtiter MPN plates, with 175 µl of *Bushnell Haas* as a growth medium per well, and 2 µl of crude oil per well as a carbon source for the bacteria. With respect to the sediments, 10 g wet weight aliquots were mixed in 90 mL of sterile detachment solution (1 g/L disodium pyrophosphate; $Na_2H_2P_2O_7$, and 20 g/L sodium chloride; NaCl) at 300 rpm for 1 hour, and the analysis was performed on the extract. The plates were incubated at 20°C for 14 days. Oil emulsion indicated positive results (Gómez-Ullate et al., 2008).

C. Crude Oil Characterization

Light Arabian crude oil supplied by Jordan Petroleum Refinery through the Munib R. and Angela Masri Institute of Energy and Natural Resources was used in the biodegradation experiments. Oil characterization was conducted to assess its physical and chemical properties, according to standard methods. The crude oil was characterized for density (ASTM 4052), API gravity (ASTM D4052), kinematic viscosity (ASTM D445), sulfur content (ASTM D4294), trace metals (Atomic Absorption), and alkanes and PAHs (Gas Chromatography – Mass Spectrometry).

D. Biodegradation Experiments

1. Microcosm Preparation

The experiments were conducted during the wet and the dry seasons of the year. In

each case, seawater and sediment samples were collected from the shoreline of Beirut, being representative of the average shoreline characteristics (table 2). The samples were contained in 250 mL silanized shake flasks rotating at 200 rpm, and at temperatures of 18°C and 28°C, representative of the peak shoreline temperature conditions attained during the wet and the dry seasons of the year, respectively. The matrix in each flask consisted of 100 mL seawater for seawater microcosms, and 100 g (wet weight) of sieved beach sediments for sediments microcosms, with 10 mL of seawater added for ease of shaking and replenished throughout the experiments as needed. Crude oil was spiked in all microcosms to achieve concentrations of 0.7 g/L of seawater and 0.7 g/kg of sediments, equivalent to 80 μL of crude oil per flask (Campo et al., 2013). The oil loading was selected based on reported amounts of crude oil spills in aquatic environments (Sammarco et al., 2013), and previous conducted studies where similar spiked amounts showed to be fully degradable within the incubation period adopted in this study (Campo et al., 2013).

2. Treatments

The batch biodegradation experiments conducted at 18°C and 28°C covered two types of biological treatments: natural attenuation to quantify the effectiveness of the intrinsic biodegradation rates, and biostimulation via the addition of nutrients, to determine the effect of nutrients enhancement on the oil biodegradation rates. For the biostimulation treatment, nitrogen and phosphorous were introduced into the corresponding flasks in the form of potassium nitrate (KNO_3) and sodium triphosphate pentabasic ($Na_5P_3O_{10}$), respectively. The final nitrogen and phosphorous concentrations in the biostimulated

microcosms approached a C:N:P stoichiometric ratio of 100:5:1 for optimal oil biodegradation rates. No additional nutrients were introduced in the natural attenuation treatment microcosms. For both treatments, oil biodegradation was carried out by the indigenous hydrocarbon-degrading bacteria present in the seawater and beach sediments, with no added exogenous cultures.

3. Procedure

11 microcosm sampling events were carried out throughout the biodegradation experiments, which lasted 42 days, a period reported to be enough to cover and monitor the complete degradation of the oil (Campo et al., 2013). The sampling events took place at days 0, 2, 4, 8, 11, 14, 17, 21, 28, 35, and 42, during which triplicate samples of seawater and sediments were sacrificed per each treatment. Therefore, a total of 132 (11×3×2×2) samples were prepared per experiment. Moreover, 3 abiotic blanks consisting of autoclaved seawater and sediments with added crude oil, and 3 killed controls (with sodium azide (NaN_3) used as sterilant at a concentration of 500 mg/L per microcosm) were prepared per each treatment to account for abiotic losses of the oil through evaporation, and removal through partitioning to the biomass, respectively. Consequently, 24 (6×2×2) additional samples were prepared, for a total of 156 (132+24) samples for each of the wet and dry season experiments. Abiotic blanks and killed controls were sacrificed during the last sampling event.

Oil extraction from the microcosms was conducted using dichloromethane (DCM) according to the methods described by Venosa et al., 2010, and Campo et al., 2013. A

surrogate solution was added into the microcosms prior to the extraction procedure on each sampling event to assess the compounds recovery rates. The surrogate solution constituents were reported elsewhere (Campo et al., 2013). For seawater microcosms, the extraction was performed using 500 mL separatory funnels, in which seawater samples were mixed with three 60 mL fresh portions of DCM and vigorously shaken for 2 minutes with periodic venting for an effective extraction. The non-miscible organic phase (solvent plus oil) and seawater were left at rest for 10 minutes for a complete phase-separation prior to the organic phase collection. The equivalent of 10 mL of sodium sulfate anhydrous were used to dry the organic extract. With respect to sediments microcosms, the extraction was performed in a soxhlet apparatus for 18-hours. The equivalent of 15 mL of sodium sulfate anhydrous were mixed with the sediment sample in the flask and placed in a thimble prior to the extraction to insure a dry sample. 250 mL of DCM were consumed per flask in the extraction process. These were used in three portions to thoroughly rinse the flask before being introduced into the soxhlet apparatus insuring the complete transfer of oil into the system. The dry organic extracts were concentrated using a rotary evaporator, then quantitatively transferred into a 25 mL volumetric flask and brought to volume with DCM.

4. Residual Hydrocarbon Analysis

The critical parameters for this study were the measured concentrations of oil components (alkanes and aromatics), which were used to determine the degradation rates and extents of the oil in the presence and absence of added nutrients. All extracts were analyzed for alkanes and aromatics by GC-MS (Agilent Technologies 7890A GC- 5975C

MSD) using an internal standard method described elsewhere (Campo et al., 2013). Alkanes included normal and branched aliphatics ranging in carbon number from 10 to 35, plus pristane, phytane, and hopane. Aromatics included 2-, 3-, 4-, and > 4-ring PAH compounds and their alkylated homologs (i.e. C0-4 -naphthalenes, C0-3 -fluorenes, C0-3 -dibenzothiophenes, C0-4 -phenanthrenes, Anthracene, Fluoranthene, C0-3 -naphthobenzothiophenes, C0-2 -pyrenes, C0-3 -chrysenes). Analyte concentrations were normalized to that of hopane, which is assumed to be non-biodegradable throughout the 42-day experiments.

E. Statistical Analysis

The biodegradation rate coefficients of individual alkanes and PAHs were examined by means of a nonlinear regression analysis using R-Studio (version 1.1.442 - © 2009-2018 RStudio, Inc). The data were fit to a simple first-order model:

$$C_1 = C_0 * \exp(-k * t)$$

where C_0 and C_1 are the initial concentration (mg/mg hopane) and concentration (mg/mg hopane) at any given time t (days) respectively, and k is the first-order biodegradation rate coefficient (day^{-1}).

Statistically significant differences were tested using Student's T-test. The null hypotheses being tested were: (1) no difference in total analyte concentrations exists between natural attenuation and biostimulation, and (2) no difference in the first order biodegradation rate coefficients of total analytes exists between natural attenuation and biostimulation.

CHAPTER III

RESULTS AND DISCUSSION

A. Crude Oil Characterization

Table 1 presents the tested oil parameters along with their respective results.

Table 1. Results of the tested crude oil parameters.

Parameters	**Results**
Kinematic Viscosity at 40 °C, cSt	76.5
Sulfur % Wt	1.25
Density at 15 °C (kg/l)	0.8804
API Gravity at 15 °C	29.22°
Metals (g/kg)	**Average** ± SD
Cadmium	<0.08 (below detection limit)
Chromium	2.12 ± 0.42
Copper	1.37 ± 0.81
Iron	23.81 ± 7.62
Vanadium	12.99 ± 0.91
Nickel	8.44 ± 0.28
Manganese	0.74 ± 0.40
Lead	0.24 ± 0.14

B. Shoreline Characterization

Table 2 summarizes the background nutrient levels in sediments and seawater samples collected form the 11 selected sites, during the dry and wet seasons.

Table 2. Summary of background nutrient concentrations by site.

	Dry Season				Wet Season			
	Total N		Total P		Total N		Total P	
Sites	**Sediment (mg/kg)**	**Seawater (mg/L)**	**Sediment (mg/kg)**	**Seawater (mg/L)**	**Sediment (mg/kg)**	**Seawater (mg/L)**	**Sediment (mg/kg)**	**Seawater (mg/L)**
Akkar	0.79±0.10	0.39±0.04	0.19±0.14	0.03±0.04	2.27±0.25	0.41±0.09	1.87±0.43	0.04±0.01
Tripoli	0.69±0.51	0.21±0.06	0.31±0.09	0.02±0.01	2.46±0.49	0.52±0.01	0.67±0.15	0.07±0.06
Chekka	0.45±0.29	0.11±0.06	0.16±0.09	0.01±0.00	2.82±0.28	0.48±0.11	0.91±0.12	0.07±0.07
Jbeil	0.94±0.35	0.21±0.05	0.07±0.01	0.01±0.01	1.22±0.03	0.41±0.02	0.38±0.02	0.06±0.01
Jounieh	0.70±0.34	0.97±0.19	0.15±0.09	0.10±0.05	2.18±0.57	0.44±0.10	0.37±0.08	0.05±0.01
Beirut	0.70±0.30	0.40±0.39	0.19±0.02	0.01±0.00	2.21±1.14	0.47±0.05	0.15±0.02	0.09±0.02
Al-Naameh	0.75±0.35	0.14±0.13	0.26±0.10	0.02±0.00	4.85±0.54	0.50±0.08	0.32±0.06	0.04±0.01
Saida	0.34±0.01	0.17±0.06	0.08±0.01	0.01±0.01	3.86±1.23	0.53±0.1	0.33±0.05	0.04±0.00
Sarafand*	1.62±0.72	0.65±0.43	0.39±0.15	0.06±0.07	-	-	-	-
Tyre	0.58±0.01	0.22±0.03	0.10±0.00	0.00±0.00	2.62±0.06	0.54±0.01	0.19±0.02	0.05±0.01
Naqoura**	0.81±0.32	0.31±0.03	0.12±0.03	0.00±0.00	-	-	-	-
Average	0.69±0.29	0.31±0.10	0.16±0.06	0.02±0.01	2.72±0.51	0.48±0.06	0.58±0.11	0.06±0.02

*** Background nutrient levels of the rocky shores of Sarafand were excluded from the shoreline average for more representative data. These shores were excluded from the wet season sampling.**
**** Access to Naqoura was prohibited during the wet season sampling for security reasons, and no results appear for this site during the mentioned season.**

The results are reported in terms of total nitrogen and total phosphorous contents. Results of nitrates, nitrites, ammonia, total kjeldahl nitrogen, and phosphate are provided in supplementary information (SI) (Table S1-4). On average, the shoreline presented measureable concentrations of total nitrogen in both beach sediments (0.69 mg-N/kg) and seawater (0.31 mg-N/L) during the dry season. These levels were significantly increased during the wet season (2.72 mg-N/kg in sediments and 0.48 mg-N/L in seawater), mainly due to the substantial increase in organic nitrogen from below detection limit in the dry season, to an average of 0.98 mg-N/kg in the wet season (Table S3). The discharge of untreated sewage in seawater partakes in maintaining such measurable nutrient levels. Moreover, the close proximity of farmlands to the shores along the shoreline, especially in the south of Lebanon (Saida and Tyr), as well as the seafront landfill in Naameh, explain

the significant increase in background nutrient levels during the wet season, due to increased agricultural and surface runoffs.

The bacteriological analysis reported average shoreline hydrocarbon-degrading bacteria concentrations of 127.47 MPN/g (wet weight) in sediments and 21.87 MPN/mL in seawater during the dry season (Table S5). Those concentrations were higher during the wet season mainly due the higher organic nitrogen content in sediments, as well as the overall higher background nutrient concentrations observed in both sediments and seawater. Those concentrations attained on average 182.97 MPN/g and 30.59 MPN/mL in sediments and seawater, respectively (Table S5). The results of the bacteriological analysis demonstrate the shoreline's intrinsic ability to mitigate oil pollution through existing hydrocarbon-degrading microbial communities. The obtained background bacterial concentrations in beach sediments were around two orders of magnitude greater than those of a similar study conducted on the shoreline of Delaware Bay (Venosa et al., 1996). The measurable background petroleum hydrocarbon levels in beach sediments along the Lebanese shoreline, ranging between 50 and 500 mg/kg (dry weight), could explain this substantial difference (Rust and Linden, 2008). Moreover, the bacterial concentrations in sediments were around 6 fold greater than those found in seawater during both seasons. This could go back to the physical properties of the matrices: the seawater matrix allows dilution and wash out of petroleum hydrocarbons, nutrients, and microoganisms by wave actions in open systems, as opposed to the sediment matrix which allows their adsorbtion and fixation.

The biodegradation experiments were conducted on samples acquired from the shore of Beirut, the capital of Lebanon, being representative of the average shoreline characteristics (Table 2 and Table S5), and the most easily accessible site.

C. Dry Season Experiments (28°C)

1. Total Alkanes and PAHs

The biodegradation of total alkanes and total PAHs at 28°C under biostimulation and natural attenuation conditions is presented in figure 3(a-d), where panels (a) and (b) depict the biodegradation of total alkanes in sediments and seawater, respectively, while panels (c) and (d) do the same with respect to total PAHs. Concentrations at any sampling event were normalized to hopane to account for losses caused by physical factors acting on the oil constituents, and ultimately to isolate the losses due to biodegradation solely (Campo et al., 2013; Venosa et al., 1996). The low water solubility and low volatility of hopanes make them persistant in the residual oil in the environment while other petroleum hydrocarbons are lost by dissolution, evaporation, and biodegradation processes, which makes them good biomarkers (Nikolopoulou and Kalogerakis, 2009; Atlas, 1995; Bragg et at., 1994). In addition, normalization to hopane eliminates potential differences in the initial loaded oil amounts in the different microcosms. Concentrations of total alkanes and total PAHs at each sampling event were calculated by summing the hopane-normalized concentrations of the individual alkane analytes (n-C_{10} to n-C_{35} plus Pristane, Phytane, and Hopane) and the individual aromatic analytes (2-ring, 3-ring, and 4-ring aromatics with their alkylated homologues), respectively, with each data point being the arithmetic mean

of three independent replicates. Omission of Non-biodegradable compounds from the analysis did not induce significant changes to the calculated biodegradation rates of both alkanes and PAHs. Hence, such omission was ultimatetly discarded to avoid inconsistencies and descrepancies when comparing the results of the dry and wet season experiments.

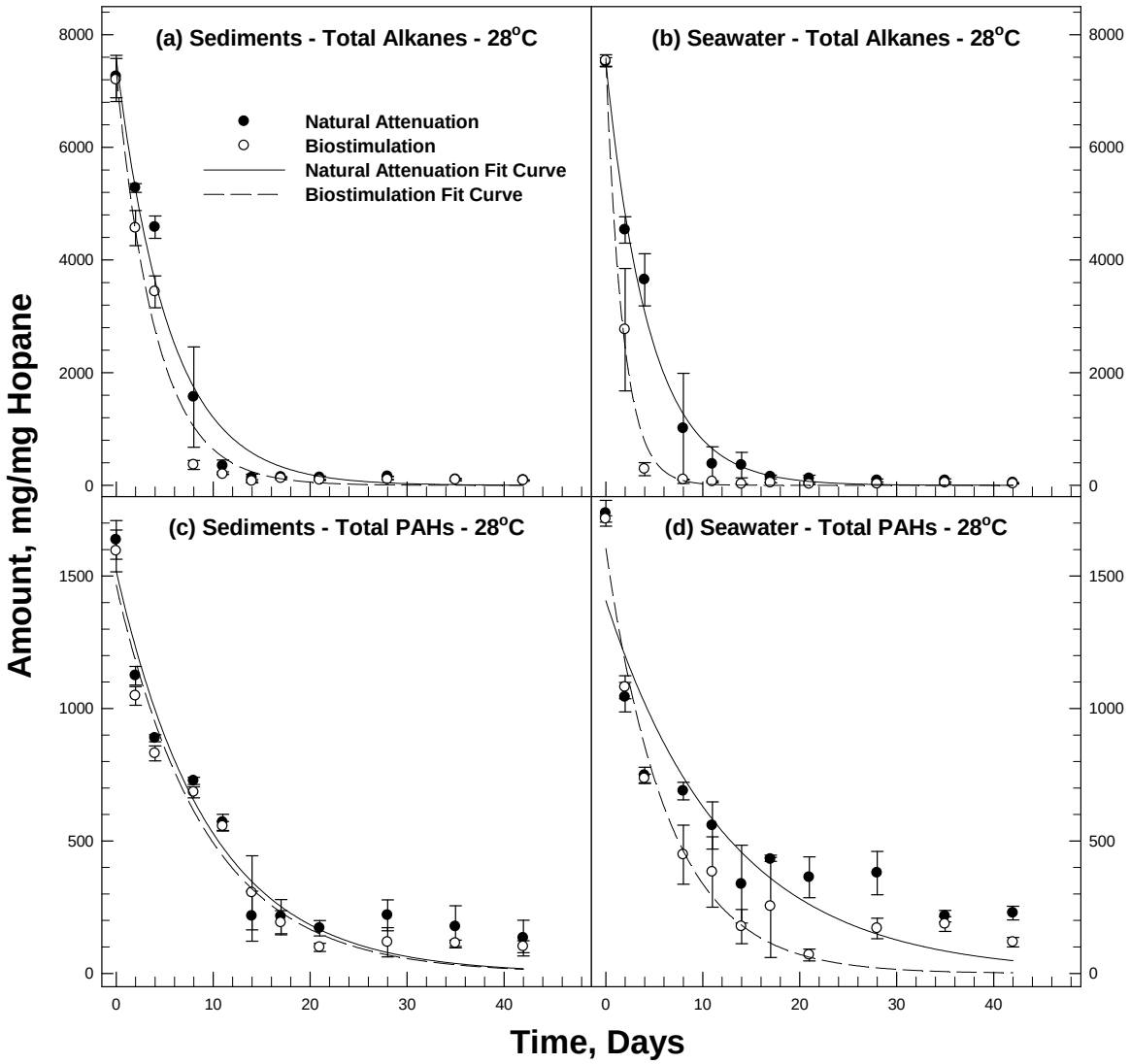

Figure 3. Biodegradation of total alkanes and total polycyclic aromatic hydrocarbons at 28°C, in marine sediments and seawater.

a. Total Alkanes

It is evident from figure 3(a) that there were significant differences in concentrations between the two treatments with respect to the biodegradation of total alkanes in sediments, confirmed by the T-test ($p < 0.05$). These differences took place at three instances: Day 2, Day 4, and Day 8, with Day 8 exhibiting the largest difference in concentrations. These differences disappeared from Day 11 onwards ($p > 0.05$), and both treatments plateaued starting Day 14 at around 1.8% residual. The results show that the natural attenuation of total alkanes was slower than the nutrient-enhanced biodegradation at first, but it caught up later on. The overall first-order rate coefficients (0.184 day^{-1} for natural attenuation, and 0.244 day^{-1} for biostimulation), show a significant ($p < 0.05$) increase of 33% in the biodegradation rate of total alkanes in sediments under the effect of biostimulation. The obtained rates in this study were significantly higher than the rates obtained under both natural attenuation (0.026 day^{-1}) and nutrient-enhanced treatment (0.056 day^{-1}) in the experimental oil spill conducted in Delaware under temperate climate, however, the degree enhancement due to biostimulation was higher in the later case (115% increase vs. 33% increase) (Venosa et al., 1996). The results of the experimental oil spill conducted in a coastal Louisiana salt marsh showed no significant improvement in the oil biodegradation rates of total alkanes under nutrient-enhanced treatments (0.0059 day^{-1} for the ammonium nitrogen addition and 0.0058 day^{-1} under for time-release urea addition) as compared to the natural attenuation rate (0.0054 day^{-1}). This could be justified by the high background nitrogen levels found in the interstitial pore water (>3 mg-N/L) as compared to the minimum nitrogen levels needed for optimal biodegradation rates. These rates, however, were far below the ones obtained in this study, which supports the assumption of

the existence of another rate-limiting factor in the coastal Louisiana salt marsh experiments, presumably the low oxygen availability (Tate et al., 2012).

Similar observations can be made in figure 3(b) with respect to the biodegradation of total alkanes in seawater, but with substantial differences in concentrations only occurring on Day 2 and Day 4 ($p < 0.05$). Day 8, Day 11, and Day 14 graphically exhibit differences in concentrations between the two treatments, however the T-test confirmed that they were not significant ($p > 0.05$). These differences disappeared from Day 17 onwards. Also, both treatments plateaued at near-complete biodegradation extents, but at different instances (Day 8 for biostimulation vs. Day 17 for natural attenuation). The overall first-order rate coefficients (0.223 day^{-1} for natural attenuation, and 0.566 day^{-1} for biostimulation), show a significant ($p < 0.05$) 1.5-fold increase in the biodegradation rate of total alkanes in seawater under the effect of biostimulation. At present, little to no literature tackle the effectiveness of biostimulation in the sea (Ron and Rosenberg, 2014), therefore, benchmarking the results of the seawater experiments in this study was not possible.

The increase in the overall biodegradation rate of total alkanes in both sediments and seawater under biostimulation conditions can be attributed to the increase in nitrogen and phosphorous levels available to the hydrocarbon degraders, which has presumably improved their metabolic activity (Das and Chandran, 2010). Based on previous literature works, it is important to assess the significance of these increases in oil biodegradation rates, and ultimately the applicability of biostimulation in the field. A review study lead by Ronald Atlas in 2009 on the bioremediation marine oil spills recommends a minimum increase in biodegradation rate by a factor of 2 (or a 1-fold increase) to consider bioremediation for mitigating marine oil spills (Atlas and Bragg, 2009). This implies that

the effect of biostimulation was powerful enough to enhance the biodegradation of total alkanes in seawater (1.5-fold increase), but not in marine sediments (33% increase). A plausible explanation to the disparate increases in rates would refer back to the background nutrient levels in both environmental matrices of the shore of Beirut summarized in Table 2. The background nitrogen level in sediments, which was equivalent to 2.8 mg-N/L of interstitial pore-water, is relatively high enough to maintain near-maximum biodegradation rates as compared to the numbers reported in the literature (2-10 mg-N/L) (Venosa et al., 2010), which left minimal room for rate-improvement via the addition of exogenous nutrients. This was not the case in seawater which had a relatively lower background nitrogen level (0.40 mg-N/L), allowing for a substantial rate improvement.

It is interesting to note that, under biostimulation conditions, the overall biodegradation rate of total alkanes was significantly higher ($p < 0.05$) in seawater as compared to their biodegradation rates in sediments. The comparison between different environmental matrices, even at very close proximities as marine sediments and seawater, can be complex, and minimal to no literature works have tackled this issue. However, factors pertaining to the physical properties of the matrix, as well as to the different existing microbial communities in each of the matrices, could give first-hand insights into the different biodegradation trends observed in sediments and seawater. Sediments, particularly fine ones, tend to adsorb nutrients on their surface. The dissolved state and the attached state of the nutrients are exchangeable via the adsorption/desorption processes, making them less bioavailable to the active microbial communities at all times (Liang et al., 2013). This entails that dissolved nutrients are more readily available to the microorganisms in seawater than in sediments, presumably explaining the faster biodegradation rates of total

alkanes observed in seawater. Moreover, oil dispersion into fine droplets in seawater microcosms under the effect of mixing increased the oil surface area for bacterial attachment, enhancing the biodegradation rates (Tyagi et al., 2011). While the partitioning of oil on sediments also increases the surface area available for bacterial activity, the limited aqueous phase in the sediment microcosms (as opposed to seawater microcosms) hindered the diffusion of the degradable hydrocarbons to the oil-water interface where their uptake by the hydrocarbon degraders occurs, leading to slower biodegradation rates (Tyagi et al., 2011; Atlas and Bragg, 2009).

b.Total PAHs

Regarding total PAHs in sediments, figure 3(c) exhibits the biodegradation curves under both treatments almost overlapping. Minor differences ($p < 0.05$) occurred at Day 2, Day 4, and Day 8. The overall trend suggests no statistically significant differences in total PAH concentrations between the two treatments from Day 11 onwards, and that the plateauing of both treatments started at Day 17 at relatively considerable residual amounts (12% residual PAHs under biostimulation condition vs. 13% under natural attenuation conditions). The first-order rate coefficients which were quite similar in magnitude (0.106 day^{-1} for natural attenuation, and 0.109 day^{-1} for biostimulation), showed a statistically insignificant ($p > 0.05$) 3% increase in rate under the impact of biostimulation. These rates were also higher than the total PAH biodegradation rates obtained in the Delaware study (0.021 day^{-1} under natural attenuation vs. 0.031 day^{-1} under biostimulation), which demonstrated however a higher degree rate enhancement through nutrient addition (50% increase vs. 3% increase) (Venosa et al., 1996).

In regards to seawater, the overall trend in figure 3(d) depicts an enhanced biodegradation rate of total PAHs in seawater under biostimulation. Both treatments however did not achieve a near-complete biodegradation extent within the 42-day period. The statistically different ($p < 0.05$) first-order rate coefficients (0.080 day^{-1} for natural attenuation and 0.156 day^{-1} for biostimulation) show around a one fold increase in biodegradation rate of total PAHs in seawater due to nutrients addition.

The same interpretation to the disparate increases in rates due to biostimulation can be applied to total PAHs as to total alkanes. The overall biodegradation rates of total PAHs however are lower than those of total alkanes. This is mainly due to the more complex structures of PAHs, consisting of two or more fused aromatic rings, as compared to the easily biodegradable straight-chain n-alkanes. The biodegradability of petroleum hydrocarbons depends on their chemical structure and their physical state. The more complex the structure is (branched and/or condensed ring structures), the more resistant it is to biodegradation, hence lower biodegradation rate. This makes PAHs more resistant to biodegradation in natural matrices (Adeniji et al., 2017; Tyagi et al., 2011; Das and Chandran, 2010; Atlas, 1995).

2. Individual Alkanes and PAHs

Figure 4(a-d) summarizes the first-order rate coefficients with respect to individual alkanes (a and b) and polycyclic aromatic hydrocarbons (c and d) at 28°C, in sediments and seawater.

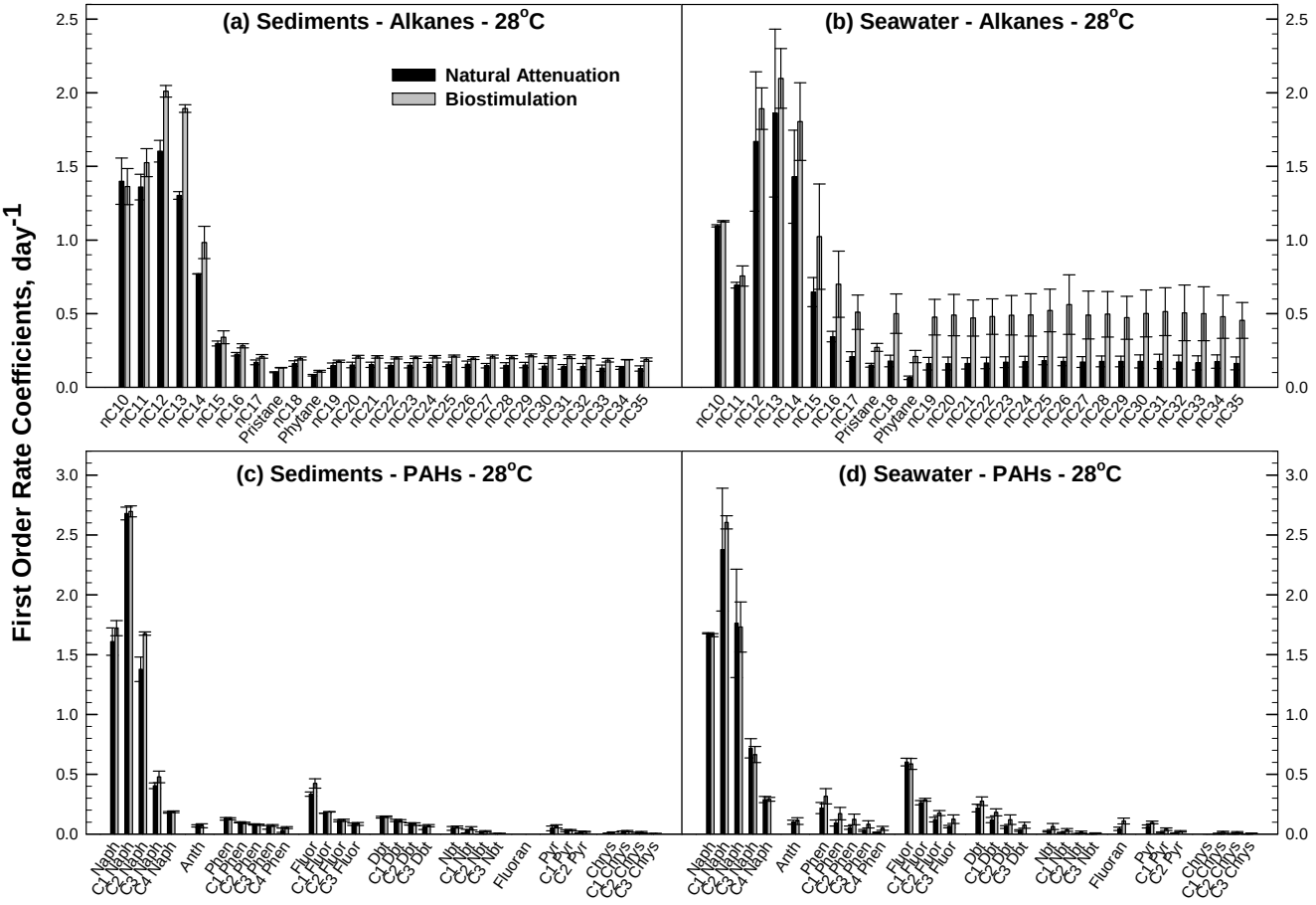

Figure 4. First-order biodegradation rate coefficients of individual alkanes and polycyclic aromatic hydrocarbons at 28°C, in marine sediments and in seawater.

a. Individual Alkanes

In sediments, the overall trend in the biodegradation of alkanes suggests a typical decrease in rate with the increase in carbon number, with biostimulation rates being slightly higher than the intrinsic rates (figure 4(a)). This trend is consistent with biodegradation behavior, as smaller hydrocarbon chains are more susceptible to biodegradation than longer chains (Venosa and Holder, 2007). Isoprenoid hydrocarbons (pristine and phytane) had relatively lower biodegradation rates under both treatments as opposed to the straight-chain

alkanes. Isopronoids are branched alkanes, which are more complex in structure than straight-chain alkanes, and therefore more resistant to biodegradation (Campo et al., 2013; Venosa et al., 1996; Atlas, 1995). Deviation from the general trend was observed in the case of the lower carbon number alkanes n-C_{10} and n-C_{11} for which lower biodegradation rate coefficients were reported. In general, n-alkanes with carbon number less than 15 can be lost by volatilization (Adeniji et al., 2017). This is expected to have reduced the initial amounts of the lower carbon chain alkanes (n-C_{10}; n-C_{11}) at early stages of the experiments setup, resulting in relatively reduced measured biodegradation rates. The first order biodegradation rate constants of the individual alkanes in the coastal Louisiana salt marsh experiments were ranging between 0.003 - 0.008 day^{-1} with respect to unbranched alkanes (n-C_{10} to n-C_{36}), and between 0.001 - 0.002 day^{-1} with respect to the branched ones (pristane and phytane). These rates are lower than the rates observed in figure 4(a) for the reasons previously discussed (Tate et al., 2012).

Similar trends can be observed in seawater (figure 4(b)), with first-order biodegradation rates in the case of biostimulation being significantly higher than those in the case of the natural attenuation treatment, explaining the biodegradation trends in figure 3(b). Exceptions in the lower carbon number alkanes were also reported (n-C_{10}; n-C_{11}; n-C_{12}).

b.Individual PAHs

The biodegradation of PAHs at 28°C exhibited similar trends in both sediments and seawater, suggesting a decrease in biodegradation rate with the increase in carbon rings and

with the increase in alkyl groups on the ring structure, which is a typical biodegradation behavior (figure 4(c-d)). Some exceptions pertaining to naphthalene occurred in both sediments and seawater, where it had lower biodegradation rates than its alkylated homologues under both treatments. This could be mainly due to its high volatility. The biodegradability of some of the 4-ring aromatics (fluoranthene, and C_0-chrysene) couldn't be properly evaluated due to their extremely low initial concentrations in oil. Overall, the biodegradation rates of PAHs in sediments were similar in magnitude under both treatments, while in seawater, rates were enhanced due to nutrient addition. This agrees with the observed biodegradation trends of total PAHs in sediments and seawater in figures 3(c) and 3(d), respectively.

D. Wet Season Experiments (18°C)

1. Total Alkanes and PAHs

The biodegradation of total alkanes and total PAHs at 18°C is summarized in figure 5(a-d), where panels (a) and (b) depict the biodegradation of total alkanes in sediments and seawater, respectively, while panels (c) and (d) do the same with respect to total PAHs.

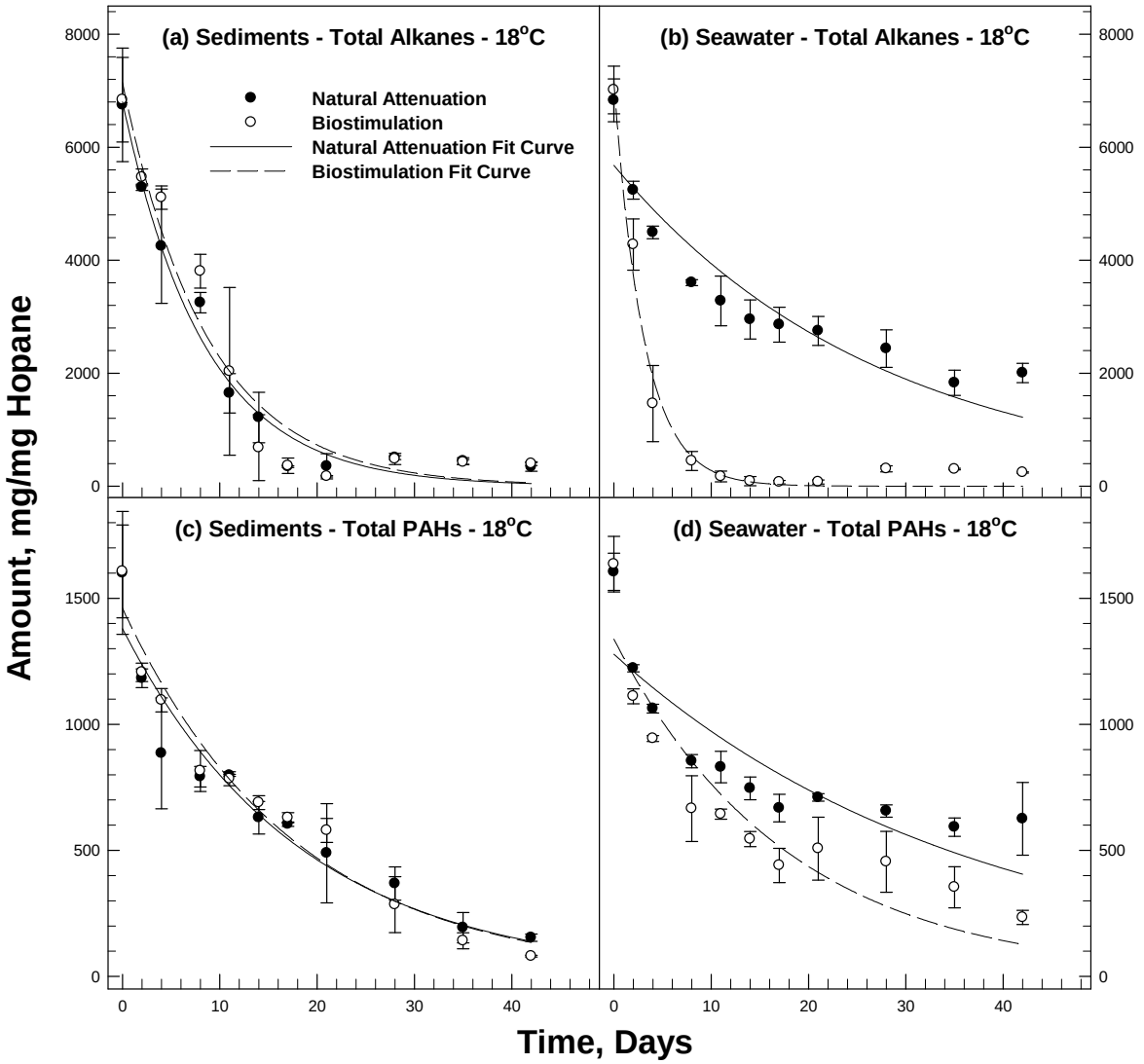

Figure 5. Biodegradation of total alkanes and total polycyclic aromatic hydrocarbons at 18°C, in marine sediments and seawater.

a. Total Alkanes

In sediments, no differences in concentrations of total alkanes between the two treatments can be observed at any sampling event ($p > 0.05$) (figure 5(a)). Also, both treatments reached near-complete total alkanes biodegradation at Day 28 (7 days behind the similar dry season experiments), and plateaued onwards. This implies that biostimulation

did not influence the biodegradation of total alkanes in sediments at 18°C. The almost equal ($p > 0.05$) first-order rate coefficients (0.119 day^{-1} for natural attenuation and 0.114 day^{-1} for biostimulation) confirm these observations. Those rates were lower in magnitude than the overall biodegradation rates of total alkanes in sediments at 28°C.

On the other hand, figure 5(b) clearly depicts the substantial effect of biostimulation in enhancing the biodegradation of total alkanes in seawater at 18°C. Significant differences in total alkane concentrations ($p < 0.05$) between natural attenuation and biostimulation treatments were measured at all instances starting Day 2. In the biostimulated microcosms, biodegradation curves plateaued at Day 11 (3 days behind the dry season experiments) at around near-complete biodegradation extent (3% residual alkanes), while 29% of the initially added alkanes amount was still available after 42 days of the biodegradation experiments under natural attenuation conditions. The first-order rate coefficient measured under nutrient enhanced conditions was equal to 0.326 day^{-1} representing an almost 8-fold increase in alkanes biodegradation rates as compared to the intrinsic value 0.037 day^{-1}. Measured biodegradation rates under both treatments were lower than their respective values reported during the dry season experiments. Figure 6 summarizes the overall biodegradation rates of total alkanes in sediments and seawater at 18°C and 28°C.

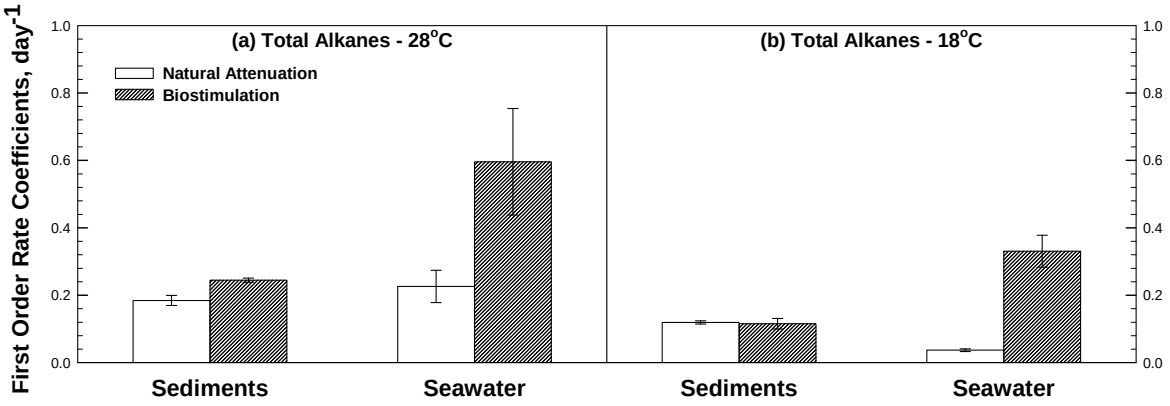

Figure 6. Summary of biodegradation rate coefficients of total Alkanes at 28°C (a), and at 18°C (b), in marine sediments and seawater.

The comparable biodegradation rates in the polluted sediments at 18°C under both natural attenuation and biostimulation treatments are associated to the high background nitrogen levels in the interstitial pore water (8.4 mg-N/L) measured during the wet season, which fall within the higher range of nitrogen concentrations necessary for maximum biodegradation rates (2-10 mg-N/L) (Venosa et al., 2010). This left no room for biodegradation rates improvement under biostimulation conditions. These background nitrogen levels were substantially higher than the nutrient concentrations measured during the dry season (2.8 mg-N/L) and where the application of biostimulation resulted in 33% rate enhancement in the biodegradation of total alkanes (Figure 6).

Contrarily to the sediments, nutrients addition exceptionally enhanced the biodegradation rate of total alkanes in seawater. The relatively low background nitrogen levels in seawater observed during the wet season on the shore of Beirut (0.47 mg-N/L) has allowed plenty of room for biostimulation to enhance the biodegradation rate of total alkanes. While measured alkanes biodegradation rate was higher in the experiments

conducted at 28°C, the effect of biostimulation was much more pronounced in seawater microcosms incubated at 18°C (1.5 vs. 8 folds increase in alkanes biodegradation rates at 28°C and 18°C, respectively) (Figure 6). This is despite the similar background nitrogen levels in seawater measured during both dry and wet seasons (0.40 mg-N/L in the dry season vs. 0.47 mg-N/L in the wet season). In general terms, biostimulation has accelerated a relatively slow biodegradation process at 18°C, while it has improved an already well-operating biodegradation at 28°C, which gives more room for rate improvements during the wet season. The lower measured biodegradation rates of total alkanes in both sediments and seawater at 18°C as compared to those measured at 28°C (Figure 6), are attributed to slower bacterial activity at the lowest temperature. Temperature plays a crucial role in the biodegradation of petroleum contaminants. It directly affects the physiology and diversity of the microbial communities, as well as the chemistry of the pollutants (Tyagi et al., 2011; Das and Chandran, 2010; Nikolopoulou and Kalogerakis, 2009). Higher temperatures (28°C) are expected to have improved the metabolic activity of the active microbial populations, inducing faster biodegradation rates. At lower temperature (18°C), bacterial metabolism is expected to have slowed down, rendering the biodegradation of pollutants slower (Das and Chandran, 2010; Zekri and Chaalal, 2007). Another plausible explanation to the exceptional total alkanes biodegradation rate enhancement in seawater at 18°C could pertain to the drastic changes in the diversity of the microbial communities biostimulation could induce. Microbial communities with microorganisms that are most capable of utilizing nutrients at the added levels will thrive in the polluted habitat, under the provided conditions (Röling et al., 2002). This implies that biostimulation in the seawater

microcosms at 18°C could have favored the growth and multiplication of the recalcitrant alkane-degraders, which resulted in the 8-fold rate enhancement.

b.Total PAHs

In sediments, the overall biodegradation trend suggests no significant differences between the two treatments at any sampling event (figure 5(c)). Moreover, the relatively slow biodegradation of total PAHs under both treatments did not reach its full extent within the studied 42-day incubation period. The almost equal overall first-order rate coefficients (0.055 day^{-1} for natural attenuation and 0.057 day^{-1} for biostimulation) further confirm these observations. These biodegradation rates were lower in magnitude than their corresponding rates in the dry season experiments.

On the other hand, figure 5(d) depicts the positive effect of biostimulation on the biodegradation of total PAHs in seawater at 18°C, where significant differences ($p < 0.05$) in PAH concentrations occurred starting Day 2, with biostimulation inducing around 1-fold increase in biodegradation rates (0.027 day^{-1} for natural attenuation and 0.056 day^{-1} for biostimulation). Both treatments were not efficient enough to ensure near-maximum degradation extent within the 42 days of the experiments.

Figure 7 summarizes the overall biodegradation rates of total PAHs in sediments and seawater at the two tested temperatures. Similar interpretations of the obtained results can be applied to total PAHs as to total alkanes. The more complex structure of the PAHs however further decreased their biodegradation rates, and further hindered biodegradation rate improvements due to nutrients addition. This explains the non-effectiveness of

biostimulation on total PAHs in sediments, and the less significant enhancement in biodegradation rate of total PAHs in seawater as compared to total alkanes.

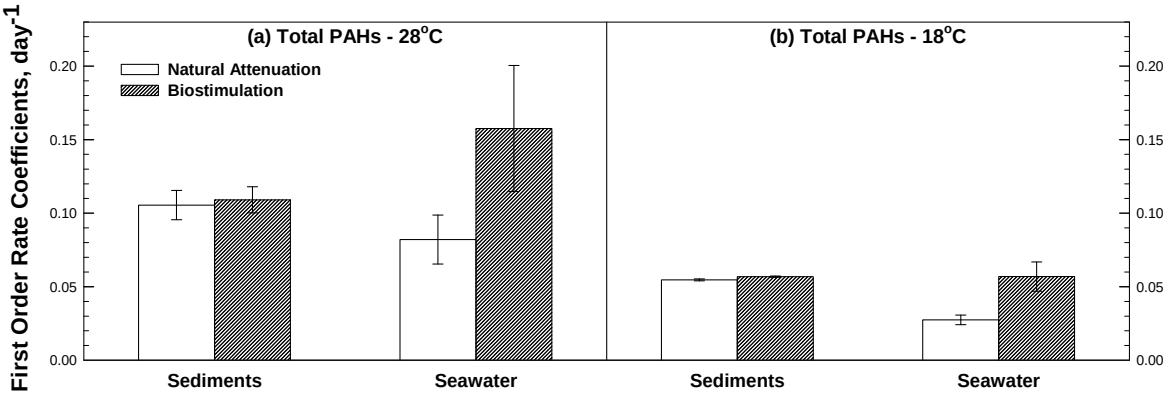

Figure 7. Summary of biodegradation rate coefficients of total polycyclic aromatic hydrocarbons at 28°C (a), and at 18°C (b), in marine sediments and seawater.

2. Individual Alkanes and PAHs

Figure 8(a-d) summarizes the first-order rate coefficients with respect to individual alkanes (a and b) and PAHs (c and d) at 18°C, in sediments and seawater.

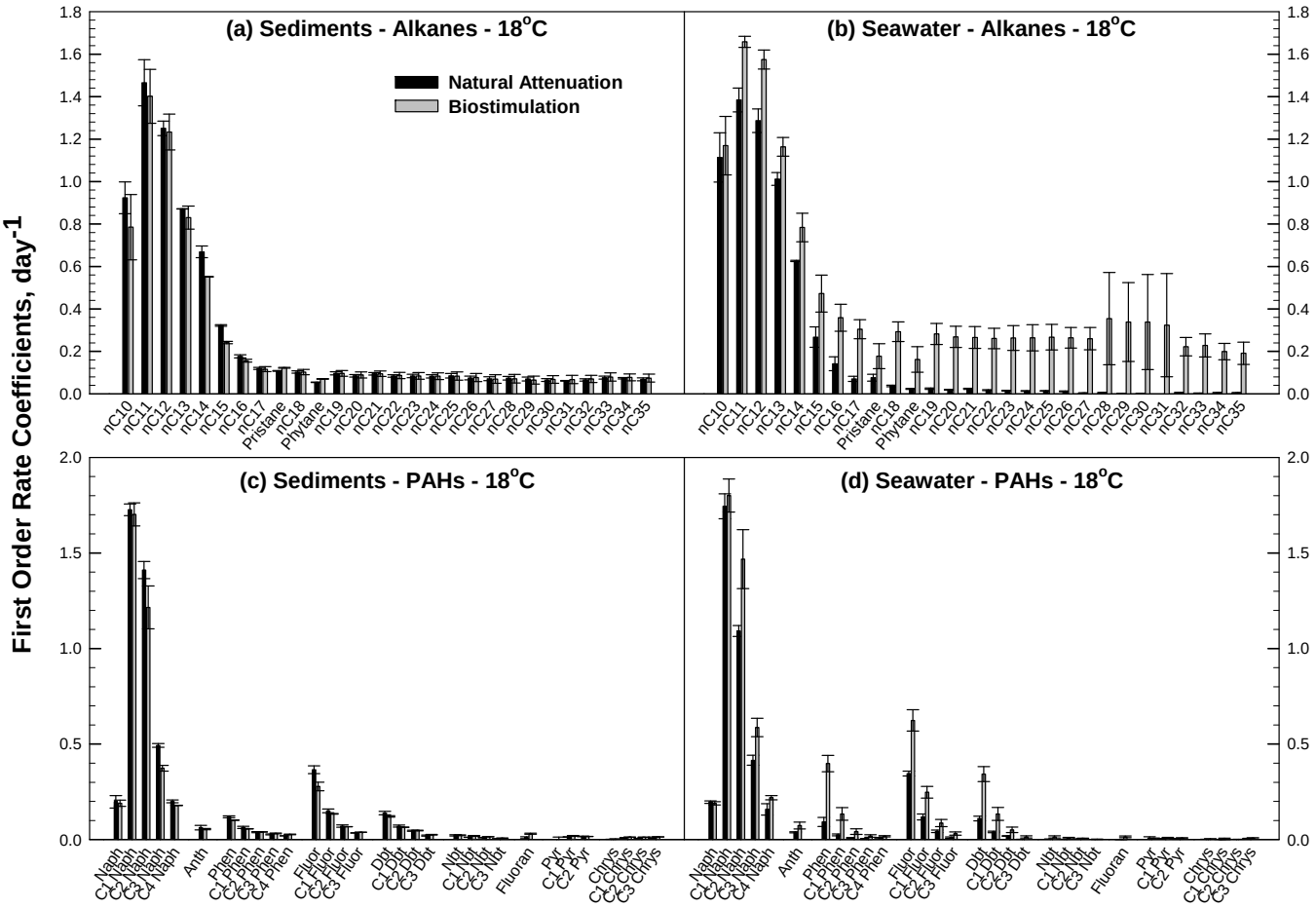

Figure 8. First-order biodegradation rate coefficients of individual alkanes and polycyclic aromatic hydrocarbons at 18°C, in marine sediments and in seawater.

a. Individual Alkanes

Similar observations to the ones previously discussed concerning individual alkanes can be made using figure 8(a and b), except that at 18°C, both treatments showed almost equal first-order rate coefficients in sediments, while in seawater, significant differences between the two treatments were expressed in the calculated biodegradation rates. Moreover, rates of both treatments in both matrices were relatively lower than the rates measured in the experiments conducted at 28°C. This further explains the slower trends in

biodegradation of total alkanes observed in the wet season experiments. The rates obtained in sediments, however, are much lower than the biodegradation rates of individual alkanes observed in a study conducted on an oil-contaminated salt marsh in Nova Scotia, Canada, at north-temperate conditions. Those individual rates ranged between 0.4 day^{-1} (for n-C_{35}) and 13.1 day^{-1} for (n-C_{10}) under natural attenuation, and between 0.9 day^{-1} (for n-C_{35}) and 42.3 day^{-1} for (n-C_{10}) under biostimulation, implying significant rate enhancements due to nutrients addition despite the high background nutrient levels observed in the field (9.5 mg-N/L and 1.2 mg-P/L in the interstitial pore water) (Garcia-Blanco et al., 2007). Reasons behind the differences between these results and the ones obtained in this study could refer to the diffrences in the geology of the contaminated sites, the properties of the affected ecosystem, the governing environmental conditions, or other biodegradation-affecting factors (Röling et al., 2002; Atlas, 1995).

b.Individual PAHs

Regarding the biodegradation of individual PAHs at 18°C, similar observations to the ones discussed at 28°C can be made using figure 8(c and d), except that at 18°C, both treatments showed almost equal first-order rate coefficients in sediments, while an overall increase in rates was noticed in seawater under the effect of biostimulation. This explains the slower trends in biodegradation of total PAHs observed in in the wet season experiments (figure 7(a and b)). The rates obtained in sediments are much lower than the biodegradation rates of individual PAHs observed in the Nova Scotia oil-contaminated salt marsh. Those individual rates ranged between 0.1 day^{-1} (for C_2-Chrysene) and 4.3 day^{-1} for

(naphthalene) under natural attenuation, and between 0.1 day^{-1} (for C_2-Chrysene) and 3.9 day^{-1} for (naphthalene) under biostimulation, indicating that no PAH biodegradation rate improvements were induced under nutrients addition (Garcia-Blanco et al., 2007).

CHAPTER IV

CONCLUSION

In summary, this study assessed the effects of biostimulation as a bioremediation technique on the biodegradation of crude oil on the Lebanese shoreline during the dry and wet seasons. It was concluded that biostimulation did not induce significant enhancement to the biodegradation rates of alkanes and PAHs where background nutrient levels were high, under the two tested temperatures. This was shown in the marine sediments of Beirut, where nutrient levels (mainly nitrogen) were high enough to ensure near-maximum biodegradation rates. These high initial nutrient concentrations are mainly attributed to the continuous discharge of untreated sewage along the shoreline, as well as the nutrient-rich runoffs from farmlands and landfills at close proximity to the shores, peaking during the wet season. In the seawater of Beirut however, initial nutrient concentrations were moderately high, but not high enough to reach near-maximum intrinsic biodegradation rates of oil constituents, which explains the significant rate enhancement biostimulation has induced during both seasons. It was also concluded that oil biodegradation rates were lower in both sediments and seawater at 18°C than at 28°C, which illustrates the temperature effect on the biodegradation of oil constituents. Bioremediation processes differ from one location to another for reasons pertaining to differences in the geology of the contaminated marine environments, the properties of the affected ecosystems, the governing environmental conditions, as well as the quantity and quality of the released hydrocarbon mixture. Finally, the present work consists of lobaratory-scale experiments conducted on closed systems (sediments and sweater microcosms) which do not ensure the replicability

of the obtained results upon the application of bioremediation strategies in open fields. Further research pertaining to the appropriate selection of the nutrient supplements formula must be conducted in order to ensure effective bioremediation, and prevent nutrient wash out and dilution in open systems. Also, the importance of the nutrient delivery systems in the field should be taken into account. Last but not least, attention should be given to the toxicity effect of bioremediation processes in the contaminated sites. The outcome of this study comprises of the results of different scenarios that could provide guidelines for policy makers and spill responders to strategically mitigate oil pollution on the Lebanese shoreline in the event of a potential crude oil spill.

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SUPPLEMENTARY INFORMATION

SI1. Chemical Analysis

a. Dry Season: Nitrogen Content

Table S1. Summary of the chemical analysis of nitrogen content during the dry season.

	Nitrate-N + Nitrite-N				Ammonia-N				Total Kjeldhal Nitogen-N				Total Nitrogen-N			
	Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)	
Site	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Akkar	0.40	0.04	0.36	0.03	0.38	0.06	0.02	0.01	BDL*	-	BDL	-	0.79	0.10	0.39	0.04
Tripoli	0.39	0.29	0.15	0.04	0.30	0.25	0.06	0.02	BDL	-	BDL	-	0.69	0.54	0.21	0.06
Chekka	0.06	0.15	0.10	0.04	0.54	0.14	0.01	0.02	BDL	-	BDL	-	0.60	0.29	0.11	0.06
Jbeil	0.58	0.36	0.13	0.01	0.37	0.10	0.08	0.04	BDL	-	BDL	-	0.94	0.46	0.21	0.05
Jounieh	0.22	0.32	0.54	0.01	0.48	0.04	0.42	0.17	BDL	-	BDL	-	0.70	0.35	0.97	0.19
Beirut	0.48	0.28	0.21	0.26	0.22	0.04	0.19	0.13	BDL	-	BDL	-	0.70	0.32	0.40	0.39
Al-Naameh	0.48	0.21	0.13	0.11	0.27	0.15	0.01	0.01	BDL	-	BDL	-	0.75	0.36	0.14	0.13
Saida	0.34	0.08	0.16	0.04	0.00	0.00	0.01	0.01	BDL	-	BDL	-	0.34	0.08	0.17	0.06
Sarafand**	0.46	0.18	0.26	0.10	1.17	0.55	0.39	0.33	BDL	-	BDL	-	1.62	0.73	0.65	0.43
Tyre	0.36	0.05	0.14	0.01	0.21	0.05	0.08	0.03	BDL	-	BDL	-	0.58	0.10	0.22	0.03
Naqoura***	0.21	0.15	0.18	0.02	0.60	0.17	0.13	0.01	BDL	-	BDL	-	0.81	0.32	0.31	0.03
Shoreline Average	**0.35**	**0.19**	**0.21**	**0.06**	**0.34**	**0.10**	**0.10**	**0.04**	**-**	**-**	**-**	**-**	**0.69**	**0.29**	**0.31**	**0.10**

*** Below Detection Limit**

**** Background nutrient levels of the rocky shores of Sarafand were excluded from the shoreline average for more representative data. These shores were excluded from the wet season sampling.**

***** Access to Naqoura was prohibited during the wet season sampling for security reasons, and no results appear for this site during the mentioned season.**

b. Dry Season: Phosphorus Content

Table S2. Summary of the chemical analysis of phosphorous content during the dry season.

	Reactive Phosphorous-P				Total Phosphorous			
	Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)	
Site	Average	SD	Average	SD	Average	SD	Average	SD
Akkar	0.19	0.14	0.03	0.04	BDL*	-	BDL	-
Tripoli	0.31	0.09	0.02	0.01	BDL	-	BDL	-
Chekka	0.16	0.09	0.01	0.00	BDL	-	BDL	-
Jbeil	0.07	0.01	0.01	0.01	BDL	-	BDL	-
Jounieh	0.15	0.09	0.10	0.05	BDL	-	BDL	-
Beirut	0.19	0.02	0.01	0.00	BDL	-	BDL	-
Al-Naameh	0.26	0.10	0.02	0.00	BDL	-	BDL	-
Saida	0.08	0.01	0.01	0.01	BDL	-	BDL	-
Sarafand**	0.39	0.14	0.06	0.07	BDL	-	BDL	-
Tyre	0.10	0.00	0.00	0.00	BDL	-	BDL	-
Naqoura***	0.12	0.03	0.00	0.00	BDL	-	BDL	-
Shoreline Average	**0.16**	**0.06**	**0.02**	**0.01**	**-**	**-**	**-**	**-**

*** Below Detection Limit**

**** Background nutrient levels of the rocky shores of Sarafand were excluded from the shoreline average for more representative data. These shores were excluded from the wet season sampling.**

***** Access to Naqoura was prohibited during the wet season sampling for security reasons, and no results appear for this site during the mentioned season.**

c. Wet Season: Nitrogen Content

Table S3. Summary of the chemical analysis of nitrogen content during the wet season.

	Nitrate-N + Nitrite-N				Ammonia-N				Total Kjeldhal Nitogen-N				Total Nitrogen-N			
	Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)	
Site	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Akkar	1.68	0.14	0.40	0.08	0.30	0.09	0.02	0.01	0.60	0.11	BDL*	-	2.27	0.25	0.41	0.09
Tripoli	1.35	0.26	0.47	0.02	0.20	0.05	0.05	0.01	1.11	0.24	BDL	-	2.46	0.49	0.52	0.01
Chekka	1.88	0.09	0.45	0.09	0.17	0.09	0.03	0.01	0.94	0.37	BDL	-	2.82	0.28	0.48	0.11
Jbeil	1.13	0.00	0.38	0.04	0.02	0.02	0.03	0.02	0.09	0.04	BDL	-	1.22	0.03	0.41	0.02
Jounieh	1.21	0.08	0.40	0.06	0.27	0.00	0.04	0.04	0.98	0.65	BDL	-	2.18	0.57	0.44	0.10
Beirut	1.32	0.05	0.45	0.07	0.03	0.00	0.02	0.02	0.88	1.19	BDL	-	2.21	1.14	0.47	0.05
Al-Naameh	1.69	0.41	0.47	0.06	0.52	0.07	0.03	0.01	3.16	0.12	BDL	-	4.85	0.54	0.50	0.08
Saida	1.55	0.22	0.50	0.09	0.42	0.07	0.03	0.01	2.33	1.47	BDL	-	3.86	1.23	0.53	0.10
Sarafand**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tyre	1.44	0.24	0.51	0.01	0.32	0.02	0.03	0.00	1.19	0.29	BDL	-	2.62	0.06	0.54	0.01
Naqoura***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shoreline Average	**1.47**	**0.17**	**0.45**	**0.06**	**0.27**	**0.05**	**0.03**	**0.01**	**1.25**	**0.50**	**-**	**-**	**2.72**	**0.51**	**0.48**	**0.06**

*** Below Detection Limit**

**** Background nutrient levels of the rocky shores of Sarafand were excluded from the shoreline average for more representative data. These shores were excluded from the wet season sampling.**

***** Access to Naqoura was prohibited during the wet season sampling for security reasons, and no results appear for this site during the mentioned season.**

d. Wet Season: Phosphorous Content

Table S4. Summary of the chemical analysis of phosphorous content during the wet season.

	Reactive Phosphorous-P				Total Phosphorous			
	Sediments (mg/kg)		Seawater (mg/L)		Sediments (mg/kg)		Seawater (mg/L)	
Site	Average	SD	Average	SD	Average	SD	Average	SD
Akkar	1.19	0.33	0.04	0.01	1.87	0.43	BDL*	-
Tripoli	0.62	0.03	0.07	0.06	0.67	0.15	BDL	-
Chekka	0.18	0.06	0.07	0.06	0.91	0.12	BDL	-
Jbeil	0.32	0.17	0.06	0.01	0.38	0.02	BDL	-
Jounieh	0.36	0.02	0.05	0.01	0.37	0.08	BDL	-
Beirut	0.00	0.00	0.09	0.02	0.15	0.02	BDL	-
Al-Naameh	0.31	0.07	0.04	0.01	0.32	0.06	BDL	-
Saida	0.22	0.01	0.04	0.00	0.33	0.05	BDL	-
Sarafand**	-	-	-	-	-	-	-	-
Tyre	0.00	0.00	0.05	0.01	0.19	0.02	BDL	-
Naqoura***	-	-	-	-	-	-	-	-
Shoreline Average	**0.35**	**0.08**	**0.06**	**0.02**	**0.58**	**0.11**	**-**	**-**

*** Below Detection Limit**

**** Background nutrient levels of the rocky shores of Sarafand were excluded from the shoreline average for more representative data. These shores were excluded from the wet season sampling.**

***** Access to Naqoura was prohibited during the wet season sampling for security reasons, and no results appear for this site during the mentioned season.**

SI2. Bacteriological Analysis

Table S5. Summary of the bacteriological analysis during the dry and wet seasons.

	Dry Season				Wet Season			
	Sediments (MPN/g)		Seawater (MPN/mL)		Sediments (MPN/g)		Seawater (MPN/mL)	
Site	Average	SD	Average	SD	Average	SD	Average	SD
Akkar	298.47	74.22	53.48	41.38	426.38	107.77	64.91	12.22
Tripoli	205.53	152.29	8.22	7.12	331.74	130.26	57.51	34.38
Chekka	116.11	119.14	3.89	6.74	219.90	144.66	57.61	19.97
Jbeil	18.36	31.79	14.02	24.28	26.10	36.91	6.13	8.67
Jounieh	0.00	0.00	6.00	0.20	27.53	38.94	8.70	4.11
Beirut	233.53	178.84	76.84	75.53	233.64	94.67	37.12	26.19
Al-Naameh	79.88	138.35	20.67	14.50	142.61	35.37	16.37	4.67
Saida	286.07	125.57	15.23	5.09	183.62	102.80	20.77	10.90
Sarafand*	361.39	85.59	206.63	99.08	-	-	-	-
Tyre	36.78	63.70	14.22	9.63	55.17	78.02	6.16	8.72
Naqoura**	0.00	0.00	6.12	1.23	-	-	-	-
Shoreline Average	**127.47**	**88.39**	**21.87**	**18.57**	**182.97**	**85.49**	**30.59**	**14.42**

*** Background microbial concentrations of the rocky shores of Sarafand were excluded from the shoreline average for more representative data. These shores were excluded from the wet season sampling.**

**** Access to Naqoura was prohibited during the wet season sampling for security reasons, and no results appear for this site during the mentioned season.**