

**Chemical characterization and toxicological assessment of photodegraded
dispersed crude oil**

by

Robyn M. Hallowell

B.S., B.A., New Mexico State University

A thesis submitted to the

Faculty of the Graduate School of the University of Colorado

in partial fulfillment of the requirement for the degree of Master of Science

Department of Civil, Environmental, and Architectural Engineering

April 17, 2015

This thesis entitled:
Chemical characterization and toxicological assessment of photodegraded dispersed crude oil
written by Robyn M. Hallowell has been approved for the
Department of Civil, Environmental, and Architectural Engineering

Karl G. Linden

Fernando Rosario-Ortiz

Joann Silverstein

Date _____

The final copy of this thesis has been examined by the signatories, and we
Find that both the content and the form meet acceptable presentation standards
Of scholarly work in the above mentioned discipline.

Abstract

Hallowell, Robyn M. (M.S., Department of Civil, Environmental, and Architectural Engineering)

Chemical characterization and toxicological assessment of photodegraded dispersed crude oil

Thesis directed by Professor Karl G. Linden

Chemical dispersants, such as Corexit 9500 and 9527, are approved by the U.S. government to reduce the environmental impact of crude oil spills on marine habitats. Such chemicals are effective as oil dispersants but the implications of their use remains largely unknown. Laboratory studies have yielded conflicting evidence regarding the toxicity of dispersed oil. Marine environments are dynamic and difficult to replicate in the laboratory; therefore, little is known about the effects of natural elements, such as sunlight, on dispersed oil mixtures. In this study, dispersed oil in artificial seawater was studied under direct sunlight and deep UV. Crude oil dispersed by Corexit is particularly difficult to characterize by analytical methods because of its tendency to form oil-dispersant emulsions. A method of extraction was developed to disrupt the emulsions formed by Corexit and extract both hydrophilic and hydrophobic fractions for chemical analysis. The chemical composition of these fractions was characterized using fluorescence spectroscopy and gas chromatography with a flame ionization detector. The degradation rates of extractable petroleum hydrocarbons and fluorescent components were calculated assuming pseudo-first order kinetics. A bioluminescence inhibition assay using the marine bacterium *Vibrio fischeri* indicated that 48 hours of equivalent sunlight exposure decreased the toxicity of dispersed oil (oil + Corexit) by 13% compared to oil only or Corexit only samples in which the toxicity increased following sunlight exposure by 8% and 6%, respectively. Additionally, mucilage extracted from prickly pear cactus, has been shown to be a non-toxic, yet effective oil dispersant. A bioluminescence inhibition assay of gelling and non-

gelling cactus mucilage extracts suggests that sunlight exposure significantly increases the bioavailability of both mucilage-dispersed oil and mucilage only samples. The results presented in this report suggest photodegradation is an important mechanism in oil spill remediation and a vital first step in improving the biodegradability of crude oil in the environment.

DEDICATION

This work is dedicated to my husband, Matt, and my son, Rowan. Their unwavering support made this possible... and worth it.

ACKNOWLEDGEMENTS

I would like to acknowledge my advisor, Professor Karl Linden, for all of his support, advice and direction throughout the course of this project. I would also like to thank Professor Fernando Rosario-Ortiz for his guidance and support. Drs. Christopher Cox (Assistant Research Professor at Colorado School of Mines), Julie Korak and James Rosenblum deserve extra thanks for helping me with my analyses. Thank you, Caitlin Glover and Stephanie Kover for helping me get this project off the ground. I would like to thank all of my lab and office mates for their kind and generous support.

Table of Contents

1	INTRODUCTION	1
2	LITERATURE REVIEW	3
2.1	Response to the largest oil spill in U.S. history	3
2.2	Corexit	4
2.3	Fluorescence spectroscopy of crude oil.....	6
2.4	Photolysis of Crude oil.....	7
2.5	Toxicity of Corexit, crude oil, and dispersed oil mixtures	8
2.6	Cactus mucilage and its usage as a natural oil dispersant	9
2.7	Summary	10
3	MATERIALS AND METHODS	11
3.1	Experimental design	11
3.2	Chemicals and reagents.....	13
3.3	Preparation of Water-Accommodated Fractions (WAF)	14
3.4	Engineered deep ultraviolet system emitting at 254 nm	14
3.5	Solar Simulator	15
3.6	Total Petroleum Hydrocarbons (TPH).....	16
3.6.1	Gas Chromatography with Flame Ionization Detector (GC-FID)	16
3.6.2	Total Petroleum Hydrocarbon (TPH) Samples	17
3.6.3	Internal and external standards	17
3.6.4	Dispersive Liquid-Liquid Extraction (DLLE)	20
3.6.5	Calculation of Total Petroleum Hydrocarbons	21
3.7	Fluorescence spectroscopy	21
3.8	Bioluminescence inhibition (BLI) assays	22
3.8.1	BLI assay of Corexit-dispersed crude oil	22
3.8.2	Cactus mucilage dispersed oil samples	23
4	RESULTS	24
4.1	Extractable Total Petroleum Hydrocarbons determined by Gas Chromatography with Flame Ionization Detector	24
4.2	Fluorescence spectroscopy of Corexit-dispersed crude oil	27
4.2.1	Fluorescence of hexane-extracted dispersed oil samples	29
4.2.2	Fluorescence of aqueous fraction of dispersed oil samples	35
4.3	Degradation of Total Petroleum Hydrocarbons by ultraviolet irradiation at 254 nm.....	37
4.4	Bioluminescence Inhibition Assays	39
4.4.1	Bioluminescence Inhibition Assay of Corexit-dispersed oil samples	39
4.4.2	BLI assay of gelling and non-gelling cactus mucilage extracts	41
5	CONCLUSIONS AND IMPLICATIONS	43
	REFERENCES.....	46
	Appendix.....	49
	Abbreviations	49

List of Tables

Table 1: Formula for 1 kilogram of 35.00% artificial seawater (Lyman and Fleming, 1940)...	13
Table 3: Components of C8-C40 alkane standard.....	19
Table 5. Half-lives of hexane-extracted fluorescent components	34

List of Figures

Figure 1: Water accommodated fraction consisting of artificial seawater, Macando crude oil, and dispersant (if appropriate).....	14
Figure 2: Spectrum of the solar simulator.....	16
Figure 3: Chromatogram for o-terphenyl (RT = 12.581 minutes, 50 ug/mL).....	18
Figure 4: GC-FID chromatogram for C8-C40 alkane standard.....	20
Figure 5: DLLE Extracts	24
Figure 6: Degradation of ETPH. Exposure time is estimated to be the natural sunlight equivalent.....	26
Figure 7: Concentration of TPH components determined by GC-FID.....	26
Figure 8: Fluorescence contour plots EEMs for hexane DLLE extracted samples (0 hours, top; 10 hours, middle; 24 hours, bottom).....	28
Figure 9: Fluorescence contour plots EEMs for aqueous DLLE extracted samples (0 hours, top; 10 hours, middle; 24 hours, bottom).....	29
Figure 10: Relative intensity of fluorescent peaks at Ex 260 (top) and Ex 240 (bottom) across all emission wavelengths.	31
Figure 11: Degradation kinetics of C1 (top), C2, C3, and C4.....	32-33
Figure 12: Relative intensity of fluorescent peaks at Ex 290 (top) and Ex 250 (bottom) cross all emission wavelengths.....	36
Figure 13: Direct photolysis of TPH extracted from Corexit-dispersed oil. Degradation rate constant = $7.78 \times 10^{-4} \text{ cm}^2 \text{ mJ}^{-1}$	38
Figure 14: Sensitized photolysis of Corexit-dispersed oil via hydroxyl radicals. Degradation rate constant = $1.05 \times 10^{-3} \text{ cm}^2 \text{ mJ}^{-1}$	38
Figure 15: Bioluminescence Inhibition (BLI) assay of Corexit dispersed oil samples.	40
Figure 16: BLI results for gelling and non-gelling cactus mucilage extract.....	42

List of Equations

Equation 1: Calculation of total petroleum hydrocarbons.....	21
Equation 2: Calculation of percent inhibition of <i>V. fischeri</i>	23

1 INTRODUCTION

The efficacy, efficiency, toxicity, and overall fate of chemical dispersants used in the clean up of oil spills have long been debated among the scientific community. On April 20, 2010 an explosion on the Deepwater Horizon drilling rig killed 11 crewmen and resulted in the largest accidental marine oil spill in history. Nearly five million barrels of Macando crude oil flowed into the Gulf of Mexico before it was capped 87 days after the initial blowout (United States Coast Guard National Response Team, 2011). As a result, a massive clean-up effort was launched to protect oceanic and wetland habitats that included the use of 1.8 million gallons of the chemical oil dispersant, Corexit (Goodbody-Gringley et al., 2013). This unprecedented use of chemical dispersants emphasized the need to better understand their formulations and the complex chemical and biological interactions that occur in the natural environment. Despite the government pre-approval and large-scale use of Corexit, there is no consensus in the scientific literature regarding its fate and toxicity to marine life, particularly under natural conditions. Also, there has been little research on the effects of weathering processes, such as photodegradation, on the fate of chemically dispersed oil. The hypotheses investigated in this study are as follows:

Hypothesis 1: Sunlight exposure causes a decrease in both the concentration of petroleum hydrocarbons and the fluorescence of dispersed crude oil.

Hypothesis 2: Sunlight exposure increases the acute toxicity of dispersed crude oil to *Vibrio fischeri*.

The research presented in this report elucidates the effects of sunlight-driven processes on the organic composition, fluorescence characteristics, and associated toxicity of chemically dispersed crude oil.

2 LITERATURE REVIEW

2.1 Response to the largest oil spill in U.S. history

On April 20, 2010 eleven people lost their lives and more than 200 million gallons of crude oil were spilled into the Gulf of Mexico when the Deepwater Horizon oil rig exploded causing a personal and environmental disaster of unprecedented magnitude. The Director of the Office of Energy and Climate Change Policy (under President Barack Obama), Carol Browner, called the spill the “worst environmental disaster the US has faced” (BBC News, May 30, 2010). Within hours of the explosion, the United States government launched initiatives to contain and clean up the oil and identify the root cause of the blast. It wasn’t until November 2012 that BP executives pled guilty to 14 criminal counts, including 11 counts of manslaughter, and agreed to a settlement of \$4 billion over five years, the largest criminal payment in U.S. history (Mufson, 2012). Despite the criminal charges and monetary settlement, both BP and the United States government received harsh criticism from media outlets and environmental groups over their respective roles in the clean up of the spill. Some say the clean up efforts were more detrimental to the environment than the spill itself (The Huffington Post,” 2014; The Guardian, 2014)); however, there is no clear consensus among the scientific community regarding the environmental impacts of spill or the mitigation efforts deployed thereafter.

Efforts to contain and mitigate the effects of the spill included the use of containment booms, incineration of floating oil, offshore filtering, and chemical dispersal. Despite these measures, oil from the Deepwater Horizon rig made its way to the shores of the Gulf of Mexico where it decimated marine and wetland habitats and forced the closure of more than 200,000 square kilometers of recreational and commercial fishing areas (“Size and Percent Coverage of

Deepwater Horizon:BP Oil Spill Closures: Southeast Regional Office,” 2010). In order to disperse the oil and protect the Gulf shoreline from further damage, more than 1 million gallons of Corexit were applied to the surface of the ocean by aircraft or boats (Hayworth, et al., 2012). Another 770,000 gallons were directly injected subsea at the source of the spill in an “experimental”, off-brand manner (On Scene Coordinator Report submitted to the National Response Team, 2011). The effects of this subsea application are still being evaluated.

2.2 Corexit

Chemical dispersants, such as Corexit, are applied to oil slicks to promote dissolution, increase the rate of biodegradation, and ultimately mineralize organic material by bacteria and plankton (Venosa, et al., 2007). Oil dispersed at the surface of the ocean also remains in direct contact with sunlight and may be subject to photochemical processes, such as direct photolysis or reaction with oxidative species such as hydroxyl radicals, which may facilitate further biodegradation. Previous work has indicated that photolytic degradation is a significant mechanism of crude oil remediation in natural environments (Brooijmans, et al., 2009; King, et al., 2014)).

Corexit is a proprietary blend of solvents and surfactants formulated by Nalco Inc. to promote dissolution and dispersal of oil in salt and brackish waters. Surfactants found in Corexit include dioctyl sodium sulfosuccinate (DOSS), Span 80, Tween 80, and Tween 85 (Glover, et al., 2014). Propylene glycol (PG), 2-butoxyethanol (2-BE), dipropylene glycol monobutyl ether (DGBE) and other petroleum distillates are the main solvent components of Corexit (“Questions and Answers | EPA Response to BP Spill in the Gulf of Mexico | US EPA,” 2010). Solvents are key constituents of any dispersant formulation as they allow the surfactants to penetrate the oil

films so that micelles can form, dispersing the oil and thus increase the rate of biodegradation (Kover, et al., 2014).

There are many processes that affect the fate and transport of Corexit and Corexit-dispersed oil in the open ocean and influence the operational effectiveness, the associated toxicity, and ultimately, the cost of clean up. Understanding these processes is important for several reasons. First, Corexit was designed specifically for use in ocean or brackish water and therefore, it is important to understand the effects of Corexit on marine life. Degradation or transformation processes may or may not affect the toxicity of oil to marine, avian, microbial, and estuarine organisms. The concern regarding toxicity is greatly exacerbated considering the large-scale application of Corexit during the DWH spill. Second, oil spill clean up is expensive and the need for low cost, yet highly effective alternatives is imperative. Many factors, such as constituent degradation, may contribute to the operational effectiveness of the applied dispersant and should be optimized for the situation at hand. Third, oil spilled in the open ocean is subject to many physical processes that ultimately affect its composition and transport. Dissolution, emulsification, evaporation, adsorption, dispersal (natural and chemically-enhanced), and photodegradation all play a significant role in the fate and transport of crude oil in the ocean (King et al., 2014).

Few studies have been completed to determine the fate of Corexit (and its components) and Corexit-dispersed oil in the environment. It has been shown that the addition of dispersants to crude oil slicks enhances microbial activity and facilitates rapid break down of oil components (Campo et al., 2013; Baelum et al., 2012; Prince et al., 2013). Surfactants in Corexit are reported to increase rates of biodegradation, influence dissolution and uptake of PAHs by microbes by attaching to the oil-water interface (Campo, et al., 2013). The photochemical fate of Corexit

constituents via direct and indirect photolysis has also been investigated. The rates of degradation reported in these studies suggest that photolysis, both direct and sensitized, may contribute to the overall fate of Corexit in the ocean (Kover, et al., 2014; Glover, et al., 2014) . To date, there have been no research studies investigating the effects of photochemical processes on Corexit-dispersed oil.

2.3 Fluorescence spectroscopy of crude oil

The use of fluorescence for the analysis of crude oil dates back more than 60 years where UV light was used to detect the presence of oil in drilling mud (Ryder, 2005) and is still used in other ways to detect oil in the natural environment.

Holmes-Smith (2012) defines fluorescence as “the radiative transfer of an electron from the first excited singlet state of a molecule back to the ground state, following absorption of a higher energy photon.” The fluorescence of crude oil is derived from the aromatic fraction that exhibits specific optical properties that are unique to each fluorescing molecule. A fluorescence signature, or “fingerprint”, may be developed for each individual type of crude oil given that the chemical composition, specifically the aromatic fraction, of different crudes varies. The use of fluorescence excitation emission matrices (EEMs) have been used previously to not only analyze the chemical composition of Macando crude oil but also the calculate the degradation half-lives of fluorescent compounds after exposure to natural sunlight. Samples of oil were exposed to sunlight, extracted with hexane, and the fluorescence was measured. As sunlight exposure increased, the fluorescence intensity, which served as a surrogate for the concentration of fluorescent compounds, decreased. The decrease in intensity followed pseudo-first order kinetics and degradation rate constants were calculated and half-lives of the components were determined (Zhou, et al., 2013).

2.4 Photolysis of Crude oil

Crude oil is a highly complex matrix that can be fractionated into asphaltenes, resins, aliphatics, and aromatic constituents. Specifically, crude oil contains long-chain alkanes and polyaromatic hydrocarbons that are typically resistant to biological and physical processes like biological hydrolysis, dissolution, or evaporation. For these recalcitrant compounds, photochemical processes are an extremely important first step in facilitating bioavailability and degradation in natural environments (King et al., 2014). Photochemical changes in oil composition can influence the ultimate fate, toxicity, and bioavailability of crude oil.

Photodegradation of crude oil may occur through direct or indirect photochemical processes. Direct photolysis occurs when a chemical absorbs photons with sufficient energy to induce changes to the chemical structure of the compound. Indirect photolysis occurs when a compound absorbs light and forms a reactive intermediate, such as hydroxyl radicals, singlet oxygen, or excited-state DOM, that interacts with the component of interest. Despite a considerable amount of research, the specific photochemical processes that take place during the weathering of crude oil are sparsely understood. Studies by Correa et al (2012) and Ray et al. (2014) indicate that exposure of crude oil to solar irradiation produces reactive photochemical intermediates, such as hydroxyl radicals and singlet oxygen. Ray et al (2014) concluded that solar irradiated crude oil is a very large source of hydroxyl radicals but barriers exist between the phases that prevent the transport of these radicals into the aqueous layer. Correa et al (2012) concluded that singlet oxygen is formed upon solar irradiation of petroleum and may be contribute to the degradation of PAHs in oil spills.

Crude oil strongly absorbs UV and visible solar radiation. Studies have shown that photolysis of crude oil is a significant mechanism of degradation in previous oil spills

(Brooijmans et al., 2009; Rojo, 2009). A study conducted by Zhou et al (2012) reports changes in the optical properties of Macando crude oil presumably caused by photolysis of crude oil components as measured by fluorescence spectroscopy. Similarly, King et al (2014) reports significant and rapid photodegradation of large PAHs upon exposure of crude petroleum to simulated solar irradiation. Furthermore, a study of Italian crude oil showed a reduction in the number of compounds identified in the water accommodated fraction after 100 hours of exposure to artificial irradiation (D'Auria, et al., 2009).

2.5 Toxicity of Corexit, crude oil, and dispersed oil mixtures

Little was known about the environmental impact of Corexit prior to DWH but retrospectively, millions of dollars have been allocated to research the fate of Corexit-dispersed oil. Currently, there is no definitive conclusion regarding the toxicity of Corexit or dispersed oil to marine, avian, and estuarine wildlife. The Environmental Protection Agency released the results of their independent study following DWH claiming that Corexit and Corexit-dispersed oil is moderately toxic to *Americamysis bahia* (mysid shrimp) and *Menidia beryllina* (inland silversides). These results indicate that neither Corexit nor dispersed oil is more or less toxic than oil alone (Hemmer, et al., 2010). A literature review by George-Ares et al (George-Ares & Clark, 2000) concluded that Corexit 9527 has low to moderate toxicity to most aquatic organisms (37 species) tested in 28 different reports. Toxicity values ranged from 1.6 ppm to >1000 ppm. However, a study by Rico-Martinez et al (2013) found that Corexit and oil separately are slightly toxic to marine rotifers (*Brachionus plicatilis*) but when combined the toxicity increases 52-fold. Researchers have also found that Corexit induces biochemical changes in marine organisms such as increased expression of heat-shock proteins, cytochrome P-450, and ethoxyresorufin O-de-ethylase (Venn, et al., 2009; Jung, et al., 2009). Toxicity assays

conducted by Paul et al. (2013) achieved conflicting results when two different microorganisms were used to assess the acute toxicity of Corexit-dispersed crude oil. The QuikLite toxicity assay, which uses a dinoflagellate, served as a proxy for phytoplankton toxicity while the Microtox assay uses luminescent marine bacterium, *Vibrio fischeri*. The dinoflagellates were less sensitive to oil than to Corexit, while Corexit was less toxic to *Vibrio* than oil alone. Paul et al. (2013) concluded *Vibrio* species are uniquely favored and that Corexit may select for *Vibrio* species in the natural environment.

2.6 Cactus mucilage and its usage as a natural oil dispersant ¹

According to a patent application filed by N. Alcantar et al. (2013), mucilage extracted from prickly pear cactus (*Opuntia ficus indica*) has shown promise as an effective, natural, and completely biodegradable oil dispersant. Cactus mucilage is a complex mixture of sugars, including galactose, rhamnose, xylose, glucose, and uronic acids that undergo several property alterations in the presence of calcium ions (similar to Corexit). When added to oil/water mixtures, mucilage facilitates the absorption of oil and dispersal of the oil film while remaining afloat. It is reported to be effective both on the surface and under the water in a manner similar to the application of Corexit in the Gulf. Cactus mucilage may be used to disperse a wide variety of petroleum-based products including crude oil, aromatics, and asphaltenes. Both gelling and non-gelling extracts are reported to have unique surface-active characteristics that enhance its ability to emulsify oils and reduce the surface tension of high polarity liquids.

¹ All information on this page was derived from a patent application filed by N. Alcantar et al. (2013); <http://www.google.com/patents/US20130087507>. Studies performed by the Alcantar group have not yet been published in peer-reviewed scientific literature.

The Alcantar group has demonstrated that both gelling and non-gelling extracts are non-toxic, but the toxicity and biodegradability of extracts in combination with oil/sea water mixtures has not been evaluated.

2.7 Summary

Despite the amount of research dedicated to characterizing Corexit-dispersed crude oil and its associated toxicity, there is no consensus among the scientific community that the measures taken to clean up the Deepwater Horizon oil spill was more or less detrimental to the environment than the spill itself. Moreover, there is little research on the effects of weathering processes on the fate, transport, and biodegradability of dispersed crude oil. The objective of this study is to appraise the effects of sunlight-driven processes on dispersed oil. Two hypotheses were considered: (1) Sunlight exposure causes a decrease in both the concentration of petroleum hydrocarbons and the fluorescence of dispersed crude oil; (2) Sunlight exposure increases the acute toxicity of dispersed crude oil to *Vibrio fischeri*.

3 MATERIALS AND METHODS

3.1 Experimental design

A series of experiments were conducted to determine the toxicological effects and photochemical fate of sunlight-degraded dispersed oil. Dispersed oil samples were made with artificial seawater, Macando crude oil, and the chemical dispersant, Corexit 9500A, or prickly pear cactus mucilage. The water-accommodated fraction, or WAF, of crude oil or dispersed oil samples was used for toxicity tests according to methods described elsewhere (Goodbody, et al., 2013; Rico-Martinez, et al., 2013; Long, et al., 2002; Hemmer, et al., 2011) and is representative of the components of crude oil that remain dispersed in the water column over time. A low-pressure mercury vapor lamp system was used to perform experiments to determine degradation rates of petroleum hydrocarbons via direct and indirect photolysis by hydroxyl radical under deep UV. A solar simulator was used to perform experiments to determine: (1) degradation rates of petroleum hydrocarbons via sunlight exposure; (2) changes in fluorescence characteristics of dispersed oil; and (3) toxicity of dispersed oil samples compared to samples of dispersant (only) and oil (only) in artificial seawater. Toxicity of exposed and non-exposed samples was determined by bioluminescence inhibition of *Vibrio fischeri*.

Dispersed oil is particularly difficult to analyze by common analytical methods because of the formation of oil-dispersant emulsions that interfere with instrumentation or extractions. These emulsions must be disrupted before extraction or chemical analysis can take place. Dispersive liquid-liquid extraction is a method of extraction developed for use in this study to disrupt the micelles formed by dispersant so that the hydrocarbons suspended in the WAF may be separated from the aqueous matrix and quantified by analytical means.

3.2 Chemicals and reagents

Hexane and boric acid (Darmstadt, Germany); acetone, hydrogen peroxide, bovine catalase, sodium chloride, magnesium chloride, o-terphenyl, strontium chloride hexahydrate, and 10x phosphate buffered saline concentrate (Sigma-Aldrich, St. Louis, MO); para-chlorobenzoic acid (ICN Biomedicals Inc., Aurora, OH); potassium chloride, sodium bicarbonate, potassium bromide, sodium fluoride, sodium sulfate, and calcium chloride (Fisher Scientific, Fair Lawn, NJ) were all of reagent grade purity. Artificial seawater was created using the recipe described by Lyman and Fleming (1940). The recipe is given in **Table 1**. Corexit 9500A was obtained from directly from Nalco Inc. (Naperville, IL). Macando Sweet Louisiana Crude oil was obtained directly from BP.

Table 1: Formula for 1 kilogram of 35.00% artificial seawater (Lyman and Fleming, 1940)

A. Gravimetric salts	
Salt	g/kg of solution
NaCl	23.926
Na ₂ SO ₄	4.008
KCl	0.677
NaHCO ₃	0.196
KBr	0.098
H ₃ BO ₃	0.026
NaF	0.003
B. Volumetric salts	
Salt	Moles/kg of solution
MgCl ₂ * 6H ₂ O	0.05327
CaCl ₂ * 2H ₂ O	0.01033
SrCl ₂ * 6H ₂ O	0.00009
C. Distilled water to 1 kg	

3.3 Preparation of Water-Accommodated Fractions (WAF)

Water accommodated fractions for oil and dispersed oil samples were prepared by mixing Macando crude oil with artificial seawater in a glass separatory funnel at a ratio of 6% (wt/wt). For dispersed oil samples, dispersant (Corexit 9500A, non-gelling, or gelling cactus mucilage) was added to samples in a 1:50 dispersant-to-oil ratio. Mixtures were gently hand shaken for ten minutes and allowed to remain undisturbed on the benchtop for one hour. After the resting period, the WAF was drained from the bottom of the separatory funnel without disturbing the layer of crude oil that remained floating on top, as shown in **Figure 1**.

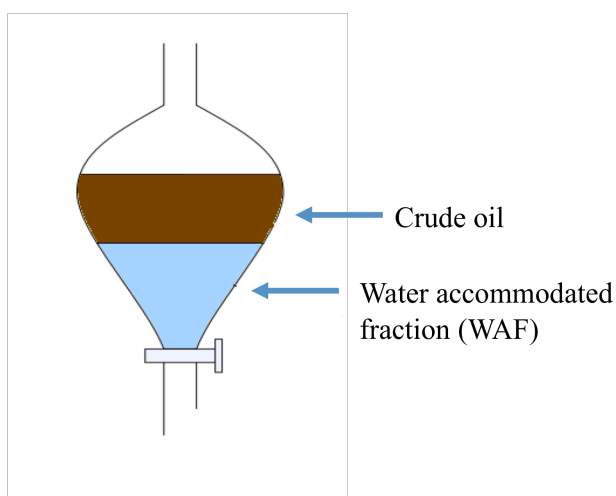


Figure 1: Water accommodated fraction consisting of artificial seawater, Macando crude oil, and dispersant (if appropriate).

3.4 Engineered deep ultraviolet system emitting at 254 nm

The engineered deep UV system used in this study is a quasi-collimated beam using a cylindrical tube (6.4 cm in diameter, 10 cm in length) and contains 4 ozone-free 15 W mercury lamps (#G15T8) that emit light at 254 nm. Samples were placed in Pyrex crystallization dishes and stirred continuously throughout the exposures. Sample absorbance, depth, and dish diameter were measured and used to calculate the average irradiance in a completely mixed batch system

according to the method developed by Bolton and Linden (2003). The sample surface irradiance was measured using an IL-1700 radiometer (Peabody, MA). Sample absorbance was measured from 200-600 nm using a Varian Cary100Bio spectrophotometer (Agilent, Santa Clara, CA). Hydroxyl radicals were produced for indirect photolysis experiments by adding 25 mg/L hydrogen peroxide to each sample. Hydroxyl radical concentrations were determined by the triiodide method described elsewhere (Klassen, et al., 1994).

3.5 Solar Simulator

Solar simulation experiments were conducted using a Model 94041 Oriel solar simulator with a 1000 W xenon lamp and an air mass 1.5 global filter (Newport Corporation, Stratford, CT). Solar simulator irradiance was measured with a spectrometer USB2000 (Ocean Optics Inc., Dunedin, FL). The irradiance was 90 W m^{-2} , which corresponded to a photon irradiance of $2.85 * 10^{-8} \text{ einstein s}^{-1} \text{ cm}^{-2}$ in the range of 290-400 nm, twice the value (44 W m^{-2}) reported for AM 1.5 global irradiance under cloudless sky when summing irradiance from 290 to 400 nm (Dong, et al., 2012). This indicates that the solar simulator was equivalent to two sun power. Samples were exposed to the beam in 2-mL glass vials with minimal headspace while lying flat in a water bath held at 20°C. The spectrum of the lamp can be seen in **Figure 3**.

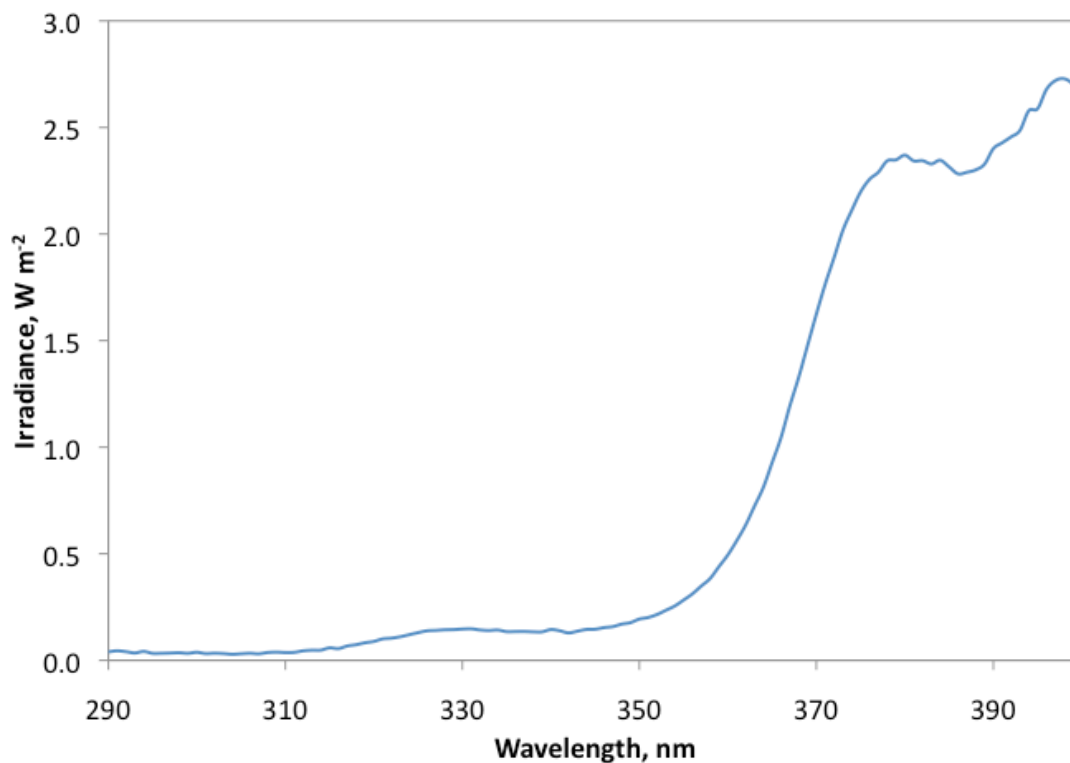


Figure 3: Spectrum of solar simulator

3.6 Total Petroleum Hydrocarbons (TPH)

The method described here is adapted from EPA method 8015C to quantify non-halogenated organics by gas chromatography and is appropriate for petroleum hydrocarbons including diesel and gasoline range organics.

3.6.1 Gas Chromatography with Flame Ionization Detector (GC-FID)

An Agilent 7890A gas chromatograph coupled with a flame ionization detector (GC-FID) and a Restek Crossbond® dimethyl polysiloxane column (20 m x 0.18 mmID, 0.18 mm film thickness). The instrument was run with the following parameters: 1 µL was injected into the inlet in splitless mode maintained at 275°C and 14.505 psi. Helium carrier gas was run through the column at a flow rate of 0.54 mL min⁻¹. The initial oven temperature was 40°C and increased

at a rate of 20°C to 325°C. The FID temperature was maintained at 350°C. Hydrogen, nitrogen (make-up flow), and air were supplied to the FID detector at rates of 35 mL min⁻¹, 30 mL min⁻¹, and 400 mL min⁻¹, respectively.

3.6.2 Total Petroleum Hydrocarbon (TPH) Samples

Samples were prepared as mentioned previously (6% wt/wt crude oil, artificial seawater, 1:50 ODR Corexit) in 2 mL vials with minimal headspace. Samples were placed in a waterbath held at 20°C beneath the beam on the solar simulator. The samples were then exposed to simulated sunlight for 3, 6, 10, or 24 hours or held in the dark (unexposed control). The samples were then added to a separatory funnel where the WAF was removed for use in dispersive liquid-liquid extraction (DLLE).

3.6.3 Internal and external standards

Internal and external standards were used to quantify extraction efficiency, response factors, and calculate the concentration of TPH. The internal standard, o-terphenyl, was chosen because it is not present in crude oil and has a high octanol/water partition coefficient ($\log P_{ow} = 5.5$) and therefore is expected to partition into the hydrophobic phase upon extraction. O-terphenyl was added to each WAF before DLLE extraction. The retention time for o-terphenyl is 12.581 minutes and the chromatogram can be seen in **Figure 5**. The extraction efficiency of DLLE extraction determined by GC-FID was greater than 95% for all DLLE extracts.

The external standard chosen for this experiment was C8-C40 alkane calibration standard (40147-U, Supelco Inc). The components in this mixture are given in **Table 3** and the chromatogram can be seen in **Figure 7**.

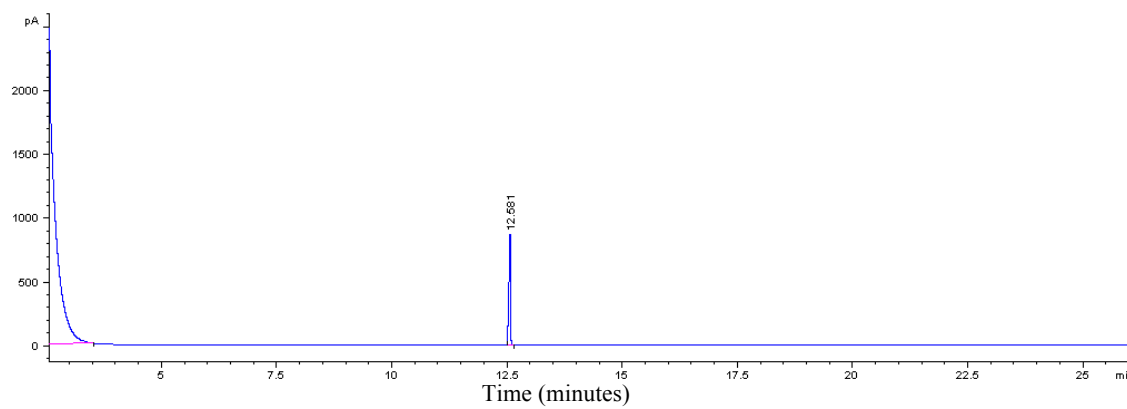


Figure 5: Chromatogram for o-terphenyl (RT = 12.581 minutes, 50 ug/mL)

Table 3: Components of C8-C40 alkane standard

Component	Concentration
Octane	500 ug/mL
Nonane	500 ug/mL
Decane	500 ug/mL
Undecane	500 ug/mL
Dodecane	500 ug/mL
Tridecane	500 ug/mL
Tetradecane	500 ug/mL
Pentadecane	500 ug/mL
Hexadecane	500 ug/mL
Heptadecane	500 ug/mL
Octadecane	500 ug/mL
Nonadecane	500 ug/mL
Pristane	500 ug/mL
Eicosane	500 ug/mL
Phytane	500 ug/mL
Docosane	500 ug/mL
Tricosane	500 ug/mL
Tetracosane	500 ug/mL
Pentacosane	500 ug/mL
Heneicosane	500 ug/mL
Hexacosane	500 ug/mL
Heptacosane	500 ug/mL
Octacosane	500 ug/mL
Nonacosane	500 ug/mL
triacontane	500 ug/mL
Hentriacontane	500 ug/mL
Dotriacontane	500 ug/mL
Tritriacontane	500 ug/mL
Tetratriacontane	500 ug/mL
Pentatriacontane	500 ug/mL
Hexatriacontane	500 ug/mL
Heptatriacontane	500 ug/mL
Octatriacontane	500 ug/mL

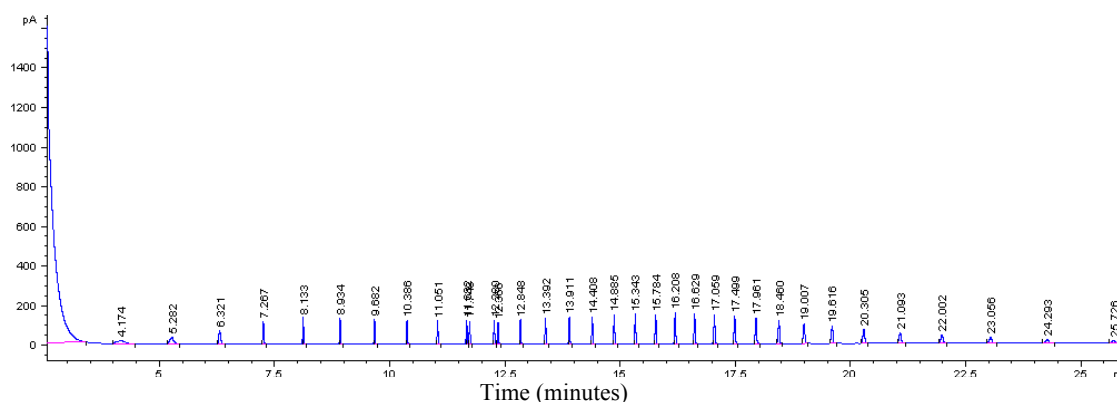


Figure 7: GC-FID chromatogram for C8-C40 alkane standard

3.6.4 Dispersive Liquid-Liquid Extraction (DLLE)

Chemical analysis of petroleum hydrocarbons present in dispersed oil samples requires the disruption of micelles formed by the dispersant and separation of the hydrophobic oil components from the hydrophilic components in the aqueous phase. A method of extraction was developed using techniques adopted from a study by Guo et al. (2011) in which polyaromatic hydrocarbons were extracted from rainwater.

Twenty milliliters of exposed and non-exposed WAF samples were placed in a separatory funnel for extraction. Ten milliliters of 50/50 mixture of hexane and acetone were added to the separatory funnel and the solution was mixed by hand for one minute with frequent venting. The mixture was allowed to rest on the benchtop for one minute before another 5 mL of acetone was added to the mixture to disrupt any additional micelles in the solution. The solution was again mixed by hand for one minute with frequent venting and then allowed to rest and separate on the benchtop for 15 minutes. The aqueous phase was pulled from the bottom and discarded. The hydrophobic fraction was drained into a glass test tube and placed under a stream of nitrogen to evaporate. The hexane was evaporated off until the extract reached a volume of 2 mL. The

fraction was then pulled from the tube using a glass pipette and placed in a glass vial. Vials were stored in the dark at 4°C until they were analyzed by GC-FID.

3.6.5 Calculation of Total Petroleum Hydrocarbons

The concentration of total petroleum hydrocarbons is determined by calculating the weight of analyte chromatographic peaks eluting in the defined retention time window and is defined by the Equation 1.

Equation 1

$$C_s = (A_x / A_s) * (C_{is} / RF) * (V_t / V_s) * D$$

Where:

C_s = Concentration of extractable petroleum hydrocarbons (mg/L)

A_x = Response for the extractable petroleum hydrocarbons in the sample (units of area)

RF = Response factor

A_s = Response for the standard (units of area, same as A_x)

C_{is} = Concentration of standard (mg/mL)

V_t = Volume of final extract (mL)

D = Dilution factor

V_s = Volume of sample extracted (L)

3.7 Fluorescence spectroscopy

Fluorescence excitation-emission matrices (EEM) were collected according to the method described by Korak et al. (2014). A Horiba FluoroMax-4 spectrofluorometer (John Yvon Horiba FluoroMax-4, NJ) was used to measure fluorescence signatures of aqueous and hexane extracted oil/dispersant mixtures. Each sample was scanned from 300 to 600 nm with 2 nm intervals under excitation wavelengths from 240 to 5500 nm in 10 nm increments. The

EEMs were collected in ratio mode with instrument-specific correction factors. Absorbance spectra were collected for each sample using a UV-Vis spectrophotometer (Cary-100, Agilent Technologies, CA) and used to correct the EEMs for primary and secondary inner filter effects. A 2 nm bandpass for excitation and emission wavelengths and 0.25 s integration time were used. The EEMs were blank subtracted and Raman normalized based on the Raman peak area for lab-grade water collected at an excitation wavelength of 350 nm. The EEMs were analyzed and corrected by MATLAB (Mathworks, MA) software and the resulting EEMs are presented in Raman Units (RU).

3.8 Bioluminescence inhibition (BLI) assays

3.8.1 BLI assay of Corexit-dispersed crude oil

Corexit-dispersed oil, oil only, and dispersant only samples were exposed to simulated sunlight for 0 (dark), 4, 8, 12, or 16 hours. Exposed and non-exposed WAF samples were prepared as described previously then used in a bioluminescence inhibition assay with *Vibrio fischeri*.

A protocol to determine the effects of sunlight exposure on the toxicity of Corexit-dispersed crude oil was adapted from Shemer et al. (2007). *V. fischeri*, a bioluminescent marine bacterium commonly used in toxicity assays, was obtained from ATCC (NRRL B-11177). Cultures were established in photobacterium broth (Fluka), grown at 20°C with continuous mixing. Cultures were harvested after 3 days by centrifuging 45 mL at 5000g for 10 minutes. The pellet was resuspended in 50 mL of 2% sodium chloride and the OD₆₀₀ was adjusted to 1.0, +/-0.05. The culture suspension was added to white, opaque 96-well plates in 100-uL aliquots. The luminescence of the culture suspension was measured using a BioTek Synergy 2

luminometer. One hundred microliters of test solution was added to respective wells in triplicate. The plates were incubated for 15 minutes in darkness before the luminescence was measured again. Positive controls (phenol or copper sulfate) were run along side each sample to ensure proper function of the assay. Test controls were conducted using artificial seawater, the same matrix from which the test samples were made. The effect of test samples on bacterial luminescence (% inhibition) was determined by **Equation 2** (Shemer et al., 2007),

Equation 2

$$\% \text{ inhibition} = 100 * \left(1 - \frac{L_t C_0}{L_0 C_t} \right)$$

where, L_0 and C_0 are the luminescence of test and control samples at time 0. L_t and C_t are the luminescence for test and control samples after 15 minutes of incubation.

3.8.2 *Cactus mucilage dispersed oil samples*

Two different extracts were obtained from Professor Alcantar's laboratory for toxicity testing; a gelling and a non-gelling extract, which are described in section 2 of this report. Mucilage extract was added to samples in an ODR of 1:50, in the same manner as Corexit-dispersed samples mentioned previously. Oil/mucilage or mucilage only samples were either exposed to 10 hours of simulated sunlight or held in the dark for the same amount of time, as described previously. The WAF was pulled off and used in toxicity testing with *V. fischeri* following the same protocol outlined in section 3.8.1 of this report.

4 RESULTS

The following section describes the results from the hydrocarbon and fluorescence degradation experiments and the bioluminescence inhibition assays of Corexit- and cactus mucilage-dispersed oil. Degradation constants for both simulated sunlight and deep UV experiments are given and the half-lives of oil components are discussed. Results of the bioluminescence assays show that sunlight may play an important role in the degradation of dispersed crude oil in the ocean.

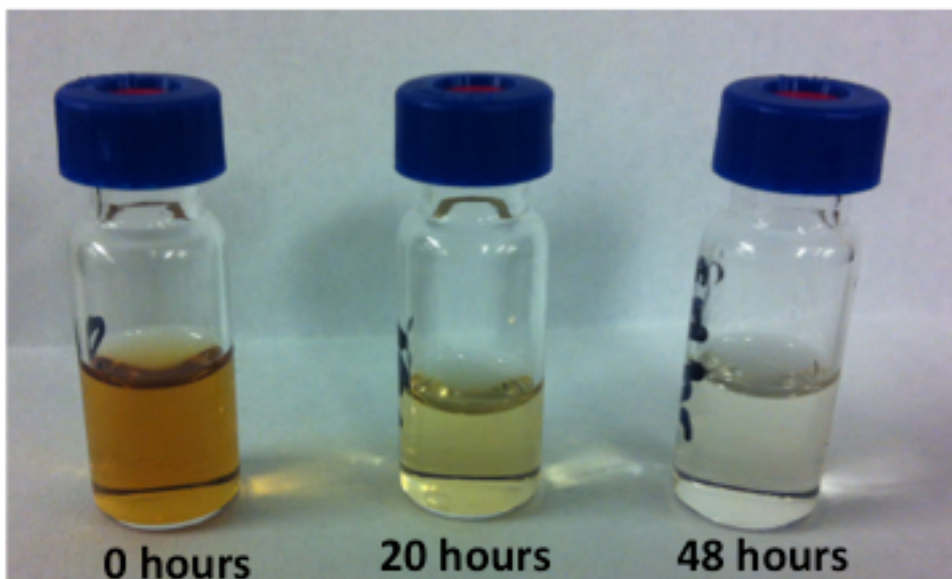


Figure 9: DLLE extracts.

4.1 Extractable Total Petroleum Hydrocarbons determined by Gas Chromatography with Flame Ionization Detector

It should be noted that all times and rate constants reported here are normalized to the natural sunlight equivalent in hours. Ten hours of simulated sunlight exposure is normalized to 20 hours of natural sunlight.

The analysis of extractable petroleum hydrocarbons by GC-FID indicates that the highest concentrations of hydrocarbons present in DLLE extracts (Figure 4) are C-10 through C-12 compounds. These compounds were identified by comparison of sample chromatograms with that of a known alkane standard. After 48 hours of sunlight equivalent exposure, the concentration of extractable total petroleum hydrocarbons (ETPH) was markedly decreased. The degradation rate constants of TPH can be calculated by fitting the natural logarithm of TPH concentration against normalized exposure time, assuming first order kinetics. The half-life of TPH under sunlight is estimated to be 8.19 hours with a rate constant of $2.35 \times 10^{-5} \text{ s}^{-1}$ (**Figure 10**). After 48 hours of sunlight equivalent exposure, the concentrations of all compounds identified by this method decreased by at least 80%, with the exception of the compounds with retention times between 8 and 9 minutes, comparable to C-10 through C-12 compounds (**Figure 12**). After 20 hours of sunlight equivalent exposure, the concentration of the C-10 through C-12 compounds decreased 37% and further irradiation did not induce additional degradation (**Figure 12**).

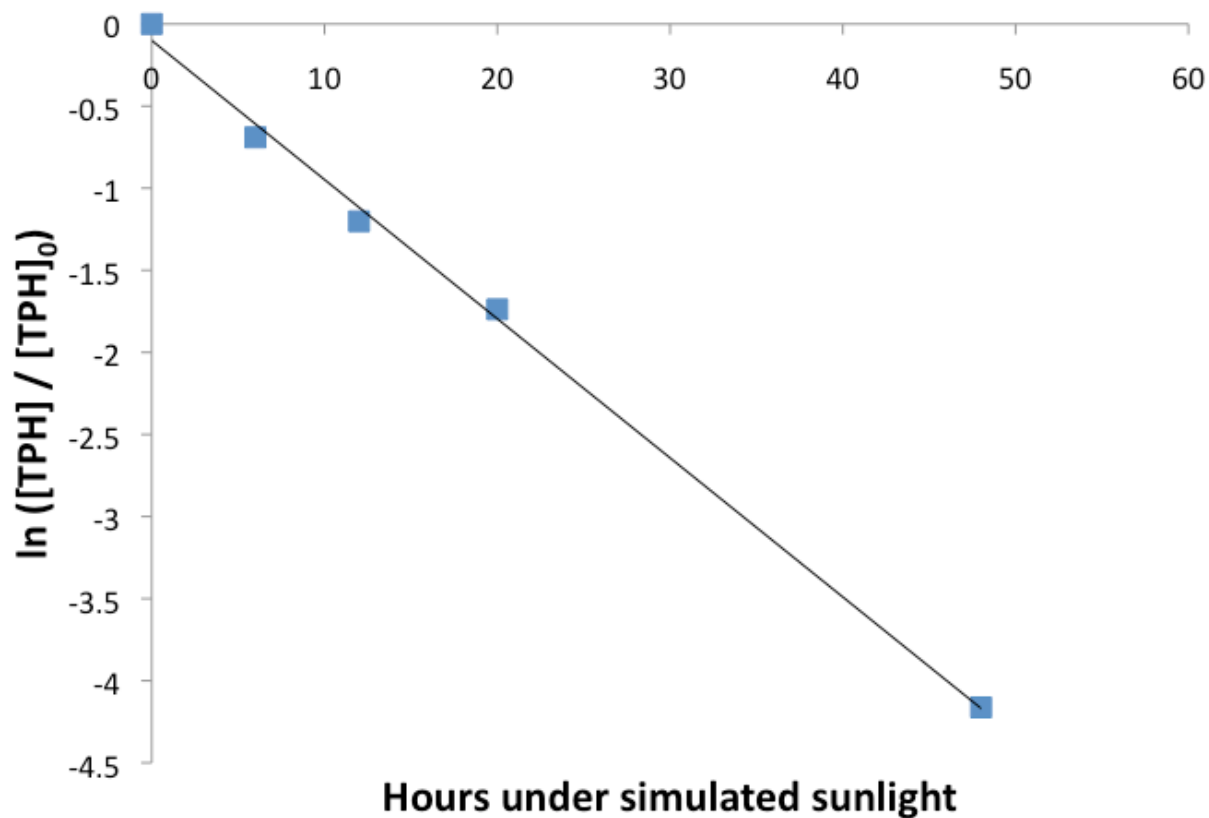


Figure 10. Degradation of ETPH. Exposure time is estimated to be the natural sunlight equivalent.

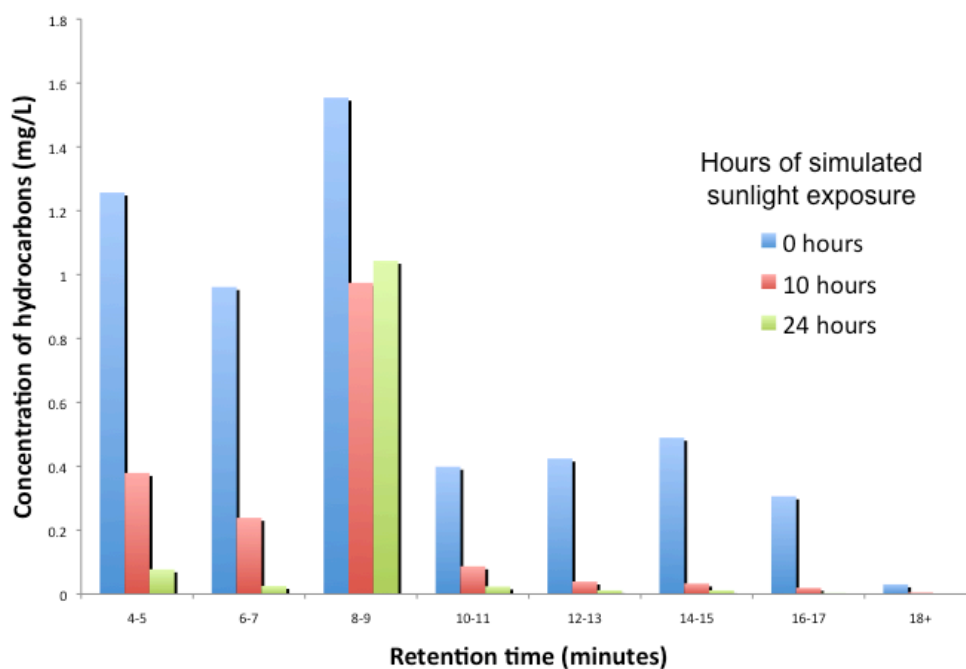


Figure 12: Concentration of TPH components determined by GC-FID

4.2 Fluorescence spectroscopy of Corexit-dispersed crude oil

The fluorescent properties of organic matter can be used to identify and fingerprint the chemical composition of crude oil because many organic compounds fluoresce at specific excitation and emission wavelengths (Bugden, et al., 2008). Contour plots generated from the EEMs for hexane-extracted and aqueous fractions of dispersed oil samples are shown in Figures 8 and 9, respectively. For the purposes of this study, peaks identified by analysis of excitation-emission matrix data are assumed to be directly proportional to the concentration of fluorescent compounds present in the extracts. This assumption is supported by literature on spectroscopy of fluorescent compounds in Macando crude oil (Zhou et al., 2013).

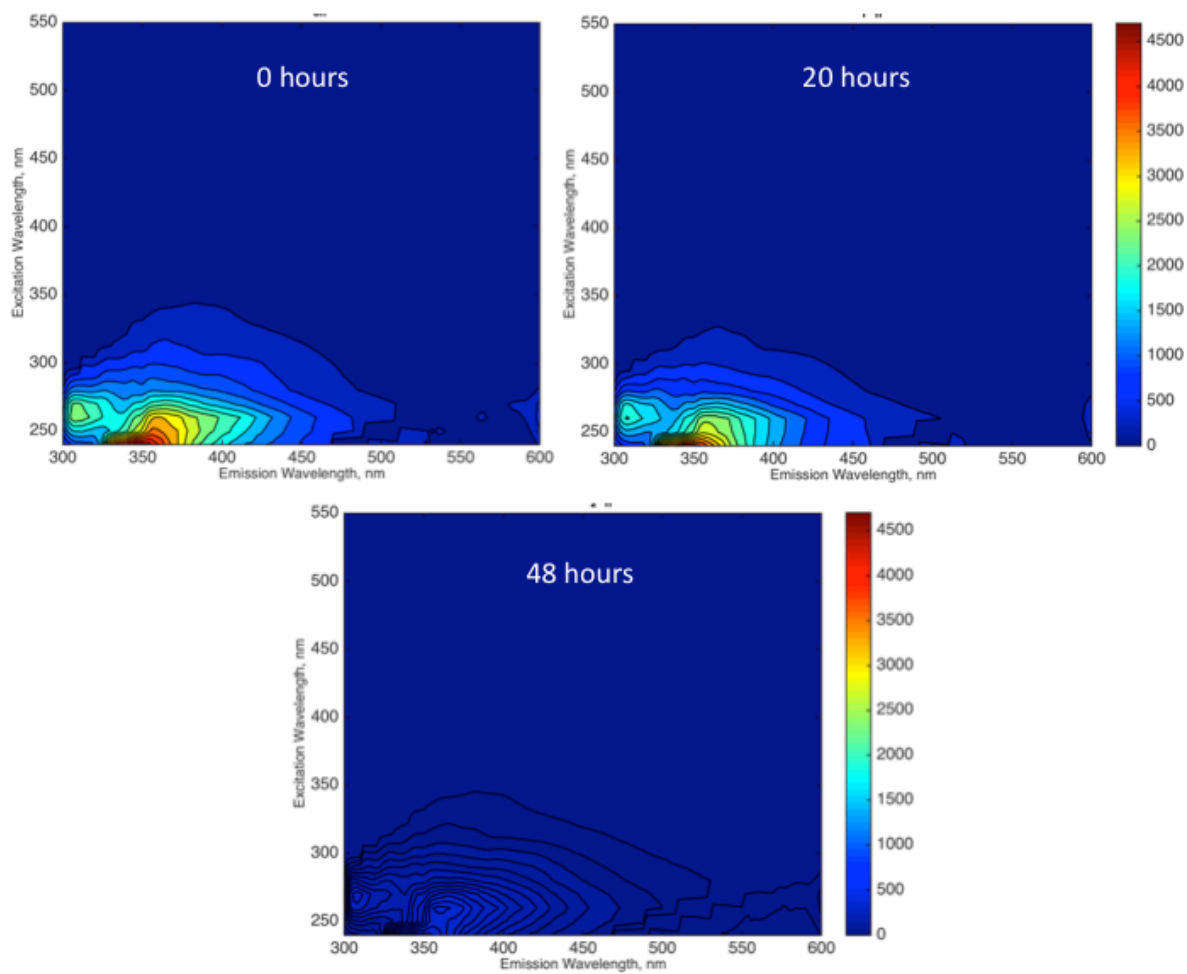


Figure 14. Fluorescence contour plots EEMs for hexane fraction DLLE extracted samples

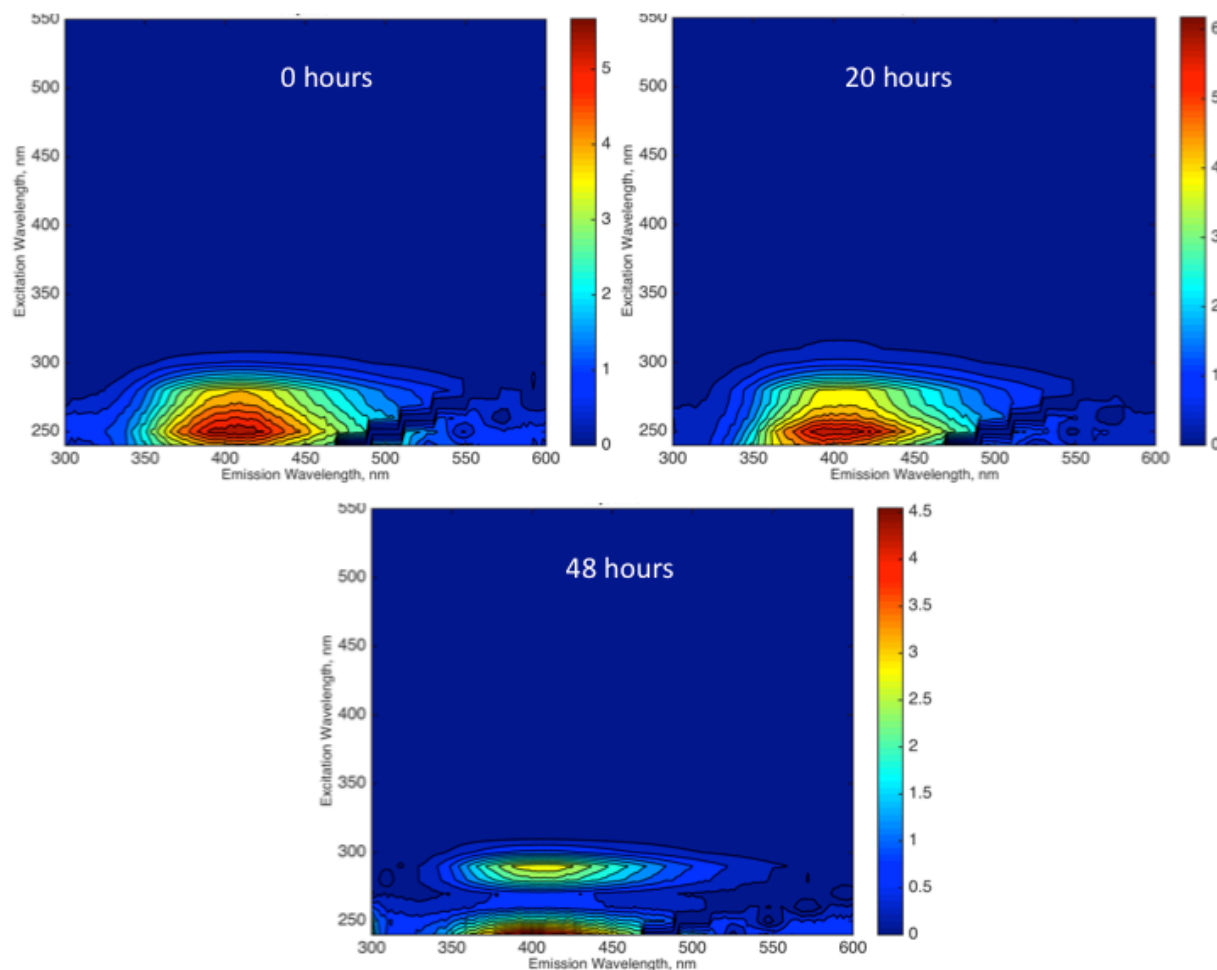


Figure 15. Fluorescence contour plots EEMs for aqueous DLLE extracted samples

4.2.1 Fluorescence of hexane-extracted dispersed oil samples

Four peaks corresponding to fluorescing molecules or specific fluorophores within larger molecules were identified in the hexane-extracted samples, each decreasing in intensity with increasing exposure to simulated sunlight. The most prominent peak appeared at Ex/Em 240/346 (C1), with smaller peaks at Ex/Em 260/308 (C2), Ex/Em 260/360 (C3), and Ex/Em 260/600 (C4) (**Figure 16**). Peak intensities at specific excitation wavelengths can be compared visually in **Figure 16**. Fluorescence intensities of all four components decreased with increasing sunlight exposure indicating photodegradation of fluorophores or fluorescing components. The

degradation rate constants of each fluorescent component can be determined by fitting the natural logarithm of fluorescence intensity against exposure time, assuming fluorescence intensity and concentration are linearly related and first order degradation kinetics (**Figure 18**). The degradation half-life under natural sunlight of each fluorescent component was estimated to 9.21, 10.58, 9.97, and 9.00 hours for C1, C2, C3, and C4 respectively. The excitation/emission wavelengths, degradation rate constants and half-lives of components identified in hexane-extracted samples are given in **Table 5**. Peak intensities at specific Ex/Em wavelengths can be visually compared in **Figure 16**.

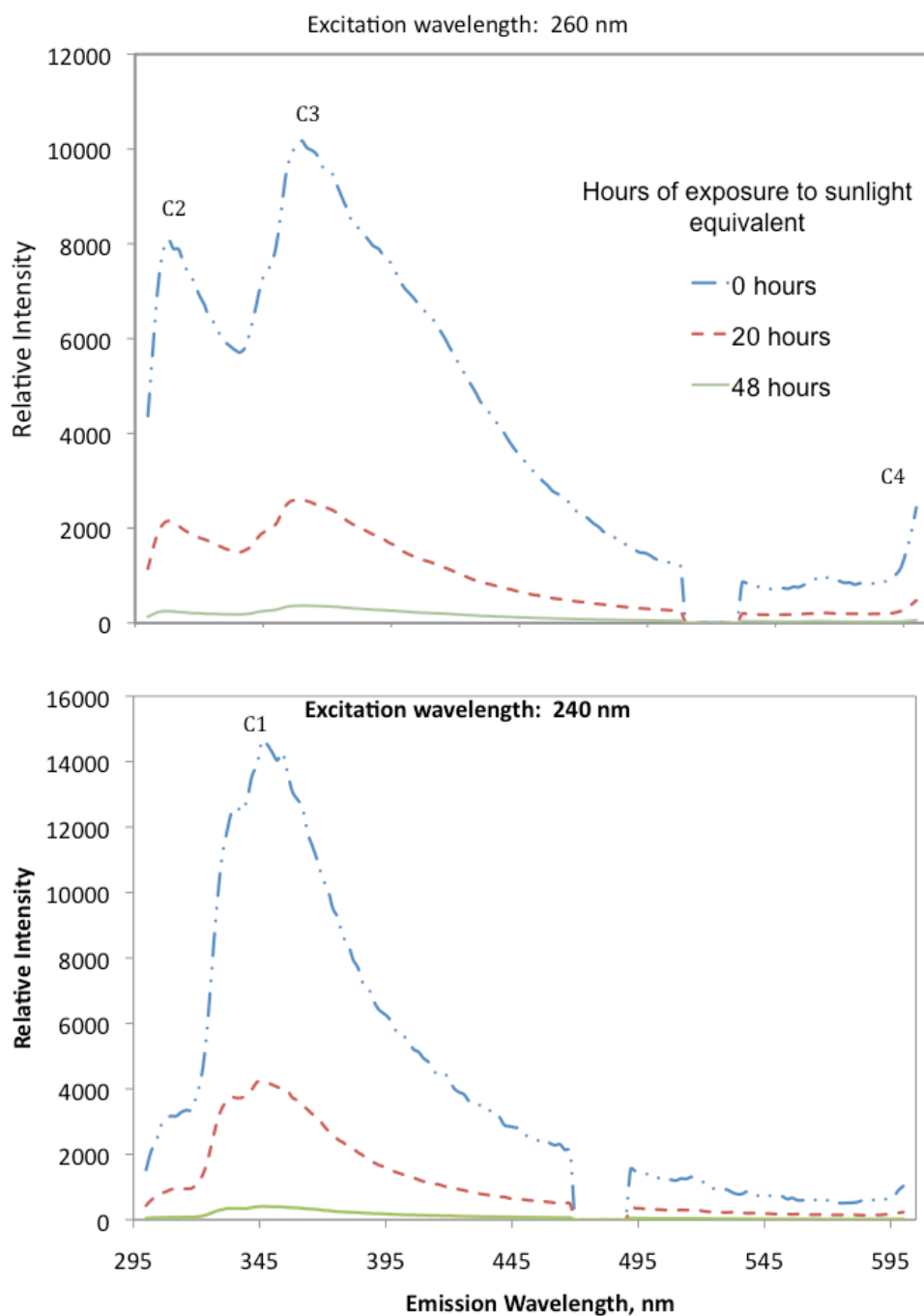
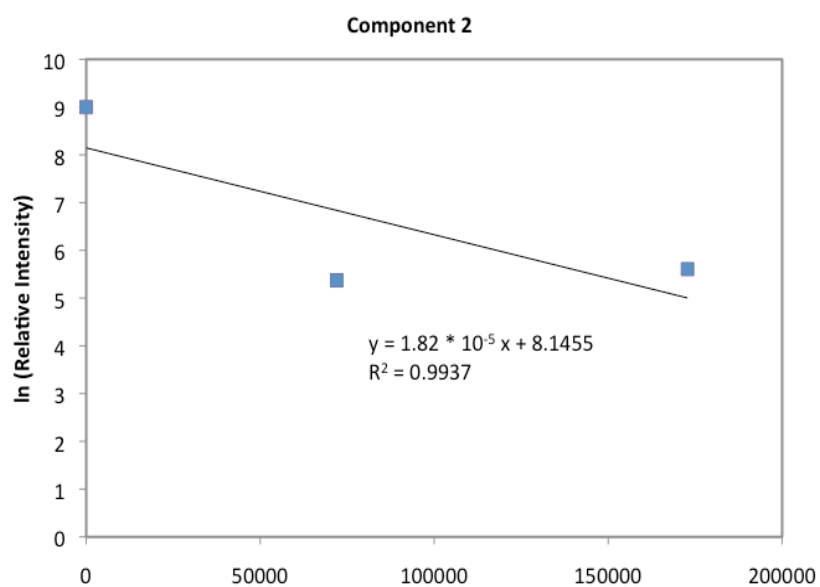
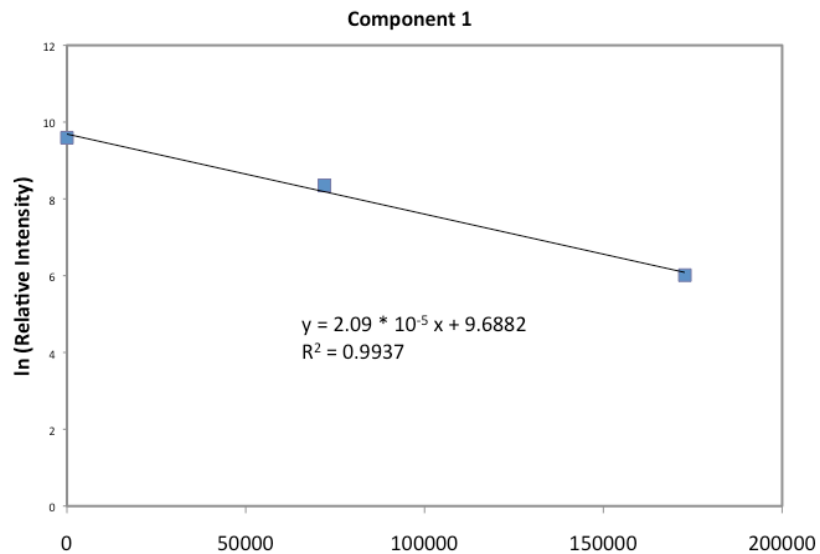


Figure 16. Relative intensity of fluorescent peaks at Ex 260 (top) and Ex 240 (bottom) across all emission wavelengths. Top graph corresponds to compounds 2, 3, and 4. Bottom graph corresponds to compound 1.



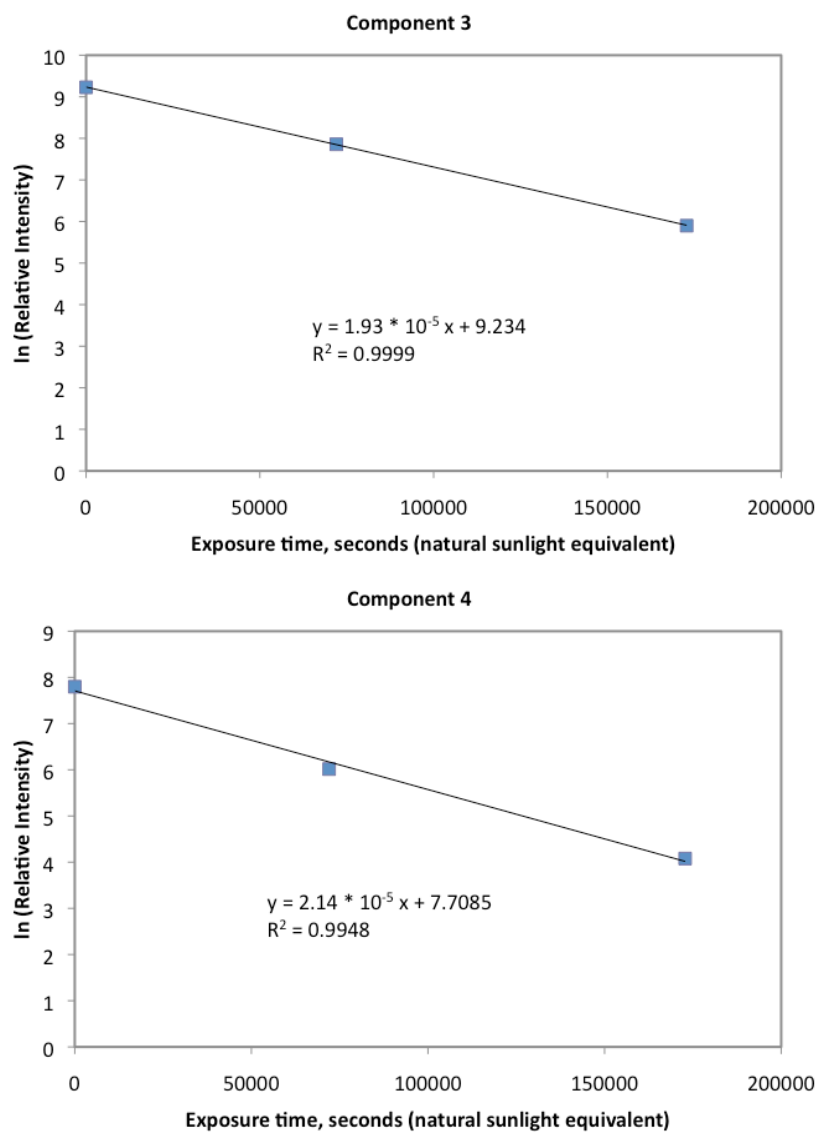


Figure 18. Degradation kinetics of C1 (top), C2, C3, and C4 (bottom).

Table 5. Half-lives of hexane-extracted fluorescent components

Component	Excitation wavelength (nm)	Emission wavelength (nm)	Rate constant (s ⁻¹)	t _{1/2} (hours)
C1	240	346	2.09 * 10 ⁻⁵	9.21
C2	260	308	1.82 * 10 ⁻⁵	10.58
C3	260	360	1.93 * 10 ⁻⁵	9.97
C4	260	600	2.14 * 10 ⁻⁵	9.00

4.2.2 Fluorescence of aqueous fraction of dispersed oil samples

The aqueous fraction that remained after DLLE extraction was examined for water soluble, fluorescent compounds. Analysis of EEM data revealed two small peaks at Ex/Em

250/408 (C5) and Ex/Em 290/408 (C6) (

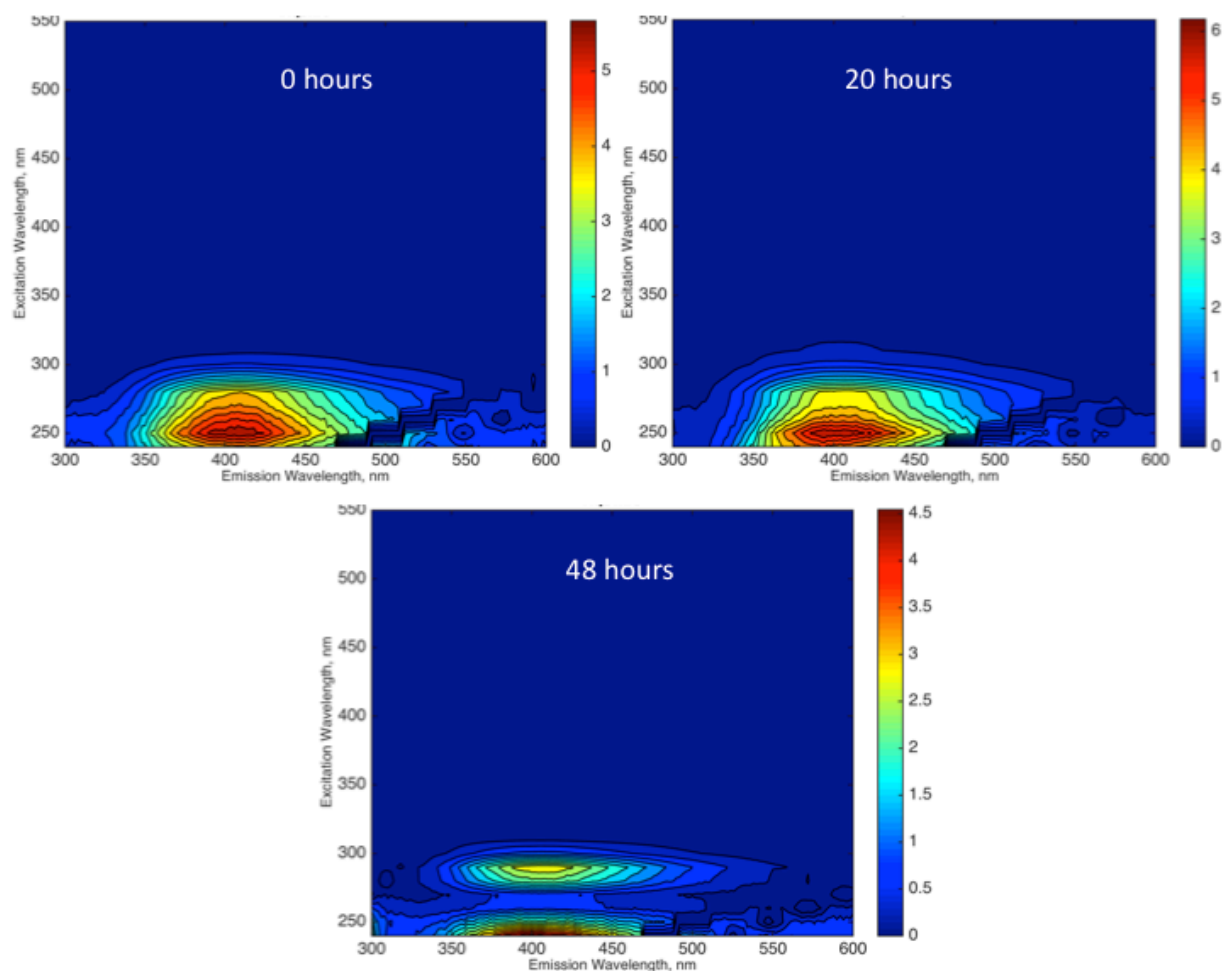


Figure 15). The intensity of the first peak, corresponding to C5, decreased with increasing simulated sunlight exposure, while the intensity of the second peak, C6, increased after 24 hours of exposure. Peak intensities at specific Ex/Em wavelengths can be compared visually in **Figure 20**.

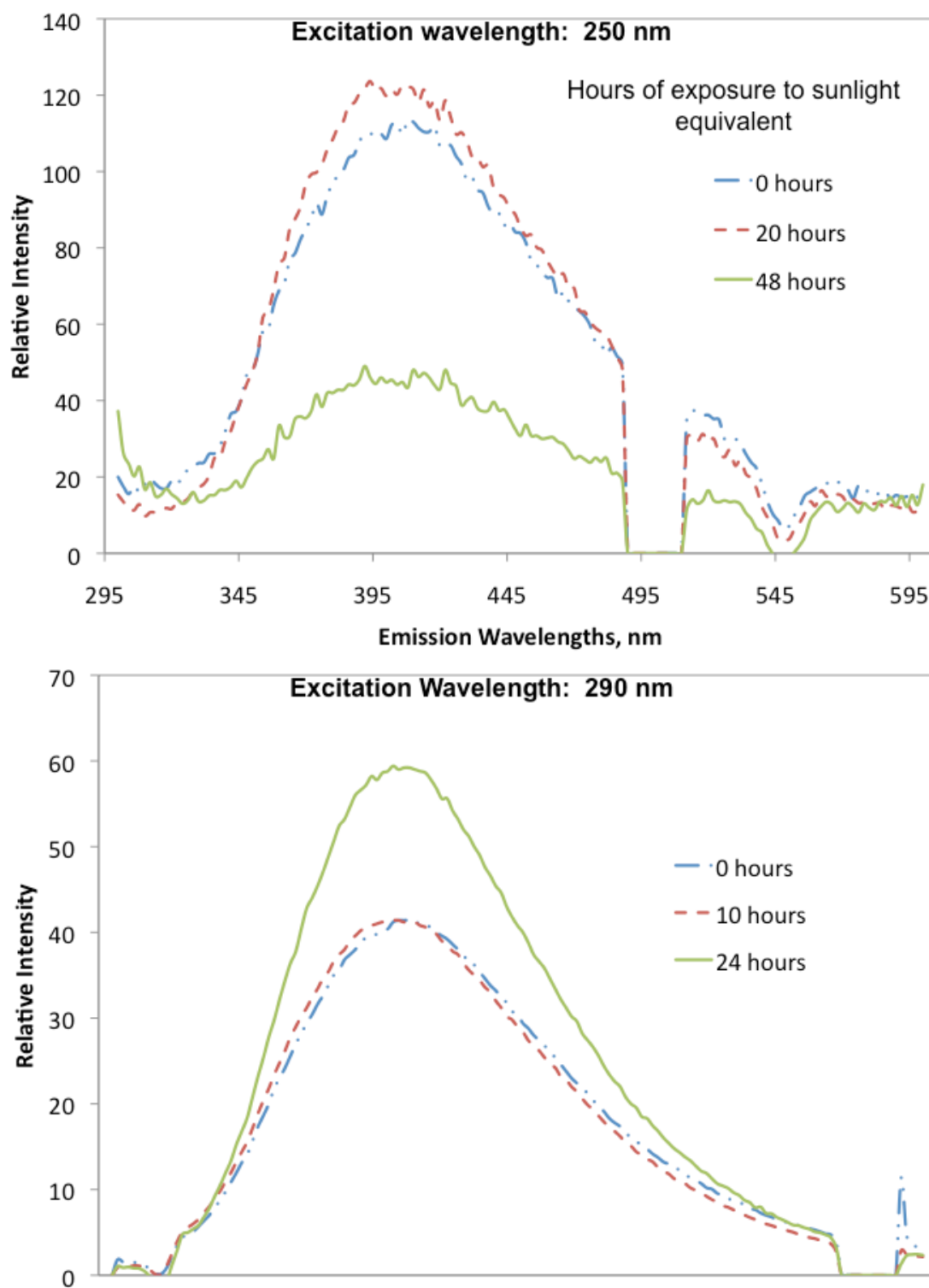


Figure 20: Relative intensity of fluorescent peaks at Ex 290 (top) and Ex 250 (bottom) across all emission wavelengths.

4.3 Degradation of Total Petroleum Hydrocarbons by ultraviolet irradiation at 254 nm

The degradation of total petroleum hydrocarbons was evaluated after exposure to direct photolysis at 254 nm and indirect photolysis via hydroxyl radicals. Figures 13 and 14 show the measured degradation of total petroleum hydrocarbons extracted from dispersed oil by direct photolysis and indirect photolysis via hydroxyl radical, respectively. The fluence-based degradation rate constants of TPH can be calculated by fitting the natural logarithm of TPH concentration against fluence, assuming first order kinetics. The degradation rate constant for direct photolysis of TPH in Corexit-dispersed oil is $7.78 \times 10^{-4} \text{ cm}^2 \text{ mJ}^{-1}$. The degradation rate for indirect photolysis via hydroxyl radicals of TPH in Corexit-dispersed oil is $1.05 \times 10^{-3} \text{ cm}^2 \text{ mJ}^{-1}$. Corexit is used primarily for marine oil spills under conditions where the water matrix contains a large number of scavengers that may affect the rate of degradation of oil components. This may explain the small difference between the rate of direct and indirect photolysis by hydroxyl radicals. In a natural system, direct photolysis likely plays a more significant role in the degradation of hydrophobic organic matter in an aqueous matrix.

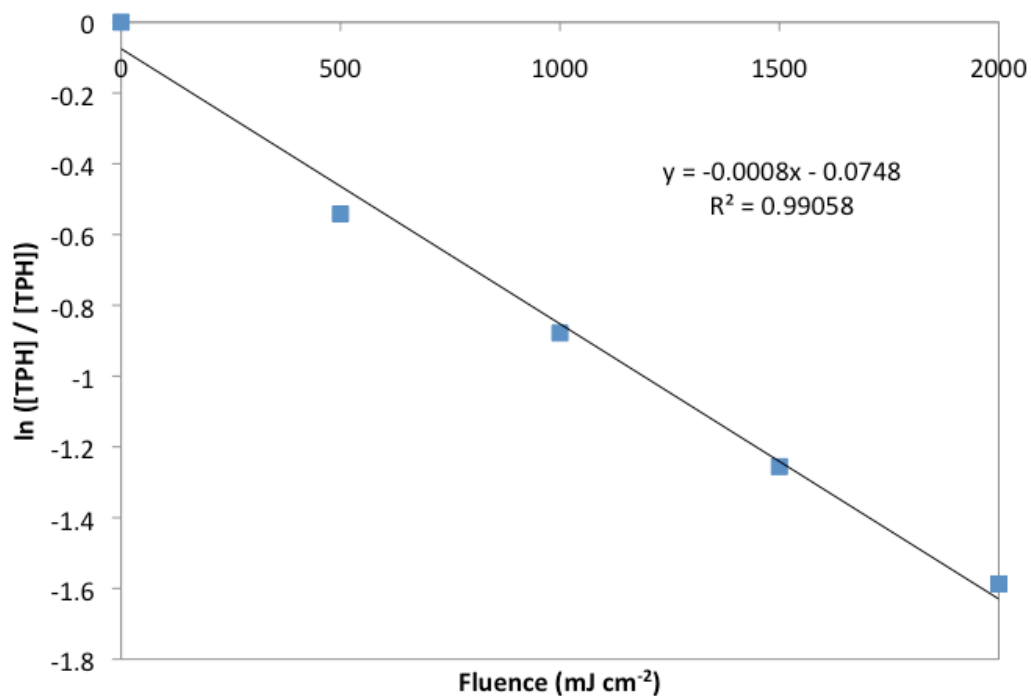


Figure 22: Direct photolysis of TPH extracted from Corexit-dispersed oil. Degradation rate constant = $7.78 \times 10^{-4} \text{ cm}^2 \text{ mJ}^{-1}$

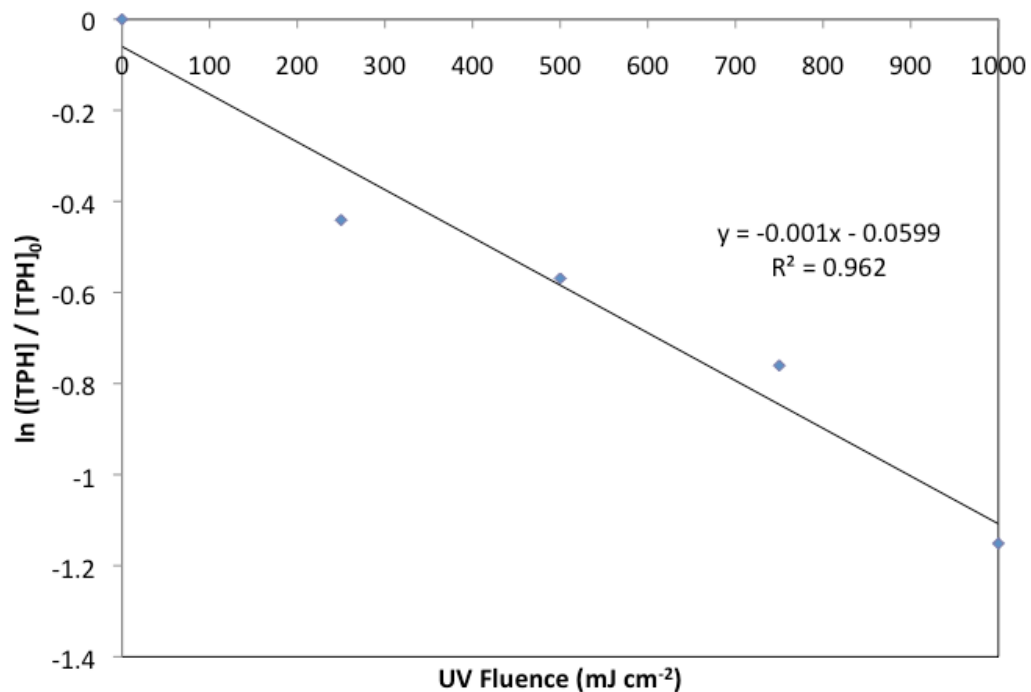


Figure 23: Sensitized photolysis of Corexit-dispersed oil via hydroxyl radicals. Degradation rate constant = $1.05 \times 10^{-3} \text{ cm}^2 \text{ mJ}^{-1}$

4.4 Bioluminescence Inhibition Assays

4.4.1 Bioluminescence Inhibition Assay of Corexit-dispersed oil samples

The bioluminescence inhibition assay is designed to measure the acute toxicity of chemicals in a water matrix using *Vibrio fischeri* as a model organism. Toxicity is measured as bioluminescence inhibition, which is directly correlated with mechanisms of cellular respiration. As a result, this assay only assesses the effects of chemicals on the cellular respiration of the bacterium. Chronic toxicity, mutagenicity and other mechanisms of toxicity are beyond the scope of the BLI assay.

Relative acute toxicity of sunlight-exposed dispersed oil, oil only, and dispersant only WAF samples was determined via bioluminescence inhibition of *Vibrio fischeri*. The water-accommodated fraction was used for this analysis for several reasons: (1) Crude oil is opaque and less dense than water. The layer of separated crude was pulled from the top of the sample so that there was no interference during the luminescence measurements (the Synergy 2 plate reader reads from the top of the plate where floating crude oil would block the light from the luminescent bacteria in the water matrix sample); (2) Crude oil separates from the water matrix over time which leads to sample inconsistency. Allowing the crude to separate and then removing it created more consistent water samples; (3) This study is investigating the effects of dispersed crude oil present in the water column (below the surface of the ocean). (4) Many other studies have reported similar methods (Goodbody, et al., 2013; Rico-Martinez, et al., 2013; Long, et al., 2002; Hemmer, et al., 2011).

The metabolic activity of the bacterial population is positively or negatively affected by constituents in the water matrix and affects the bioluminescence accordingly. Inhibition of luminescence is indicative of toxicity while amplified luminescence is indicative of activity. The

results of the BLI assay indicate that bioluminescence of *Vibrio fischeri* significantly increased when exposed to Corexit-dispersed Macando crude oil samples. Thirty-two hours of equivalent sunlight exposure decreased the toxicity of dispersed oil (oil + Corexit) by 13% (+/- 2.6%) compared to oil only samples in which the toxicity increased by 8% (+/- 1.6%) following sunlight exposure. Sunlight-induced toxicity was demonstrated by bioluminescence inhibition of exposed crude oil samples while exposure of dispersant only samples had no effect on the bacterial population. Results of the BLI assay, seen in **Figure 24**, indicate that sunlight exposure may reduce the toxicity of dispersed oil and subsequently increase the bioavailability. These results are contrary to the original hypothesis set forth in this study.

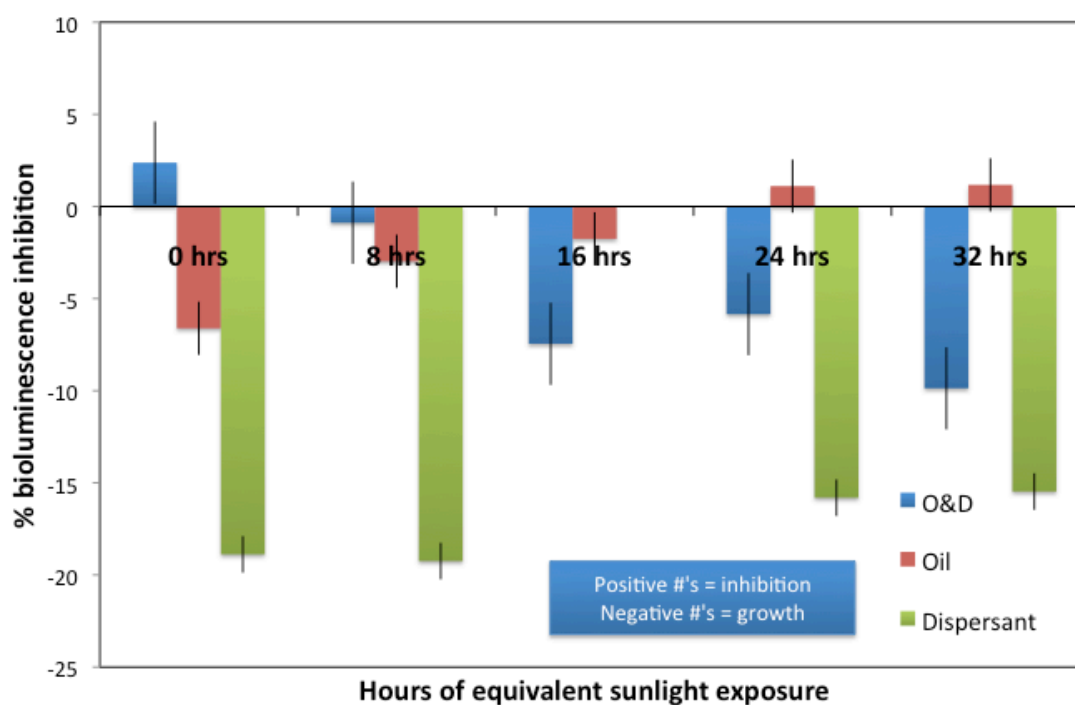


Figure 24. Bioluminescence Inhibition (BLI) assay of Corexit dispersed oil samples. The error bars represent the relative standard error for the respective samples in the assay (2.6% for dispersed oil samples, 1.6% for oil only samples, 0.8% for dispersant only samples).

4.4.2 BLI assay of gelling and non-gelling cactus mucilage extracts

Relative toxicity of cactus mucilage-dispersed oil was determined via bioluminescence inhibition of *Vibrio fischeri* in a manner similar to the BLI assay performed with Corexit. Gelling and non-gelling cactus mucilage extracts were mixed with oil/seawater or seawater only in a 1:50 oil-to-dispersant ratio. Samples were exposed to sunlight or held in the dark for the same amount of time then subjected to the BLI assay using *V. fischeri* as a test organism. Results from the BLI assay show negative inhibition of all samples indicating an increase in bioluminescence of *V. fischeri* in all samples tested. Sunlight exposure caused a bioluminescence increase of 20.9% for samples containing gelling extract and crude oil and an 11.5% increase for samples containing non-gelling extract and crude oil. Sunlight exposure also increased the bioluminescence of *V. fischeri* by 8.9% and 8.4% when treated with gelling and non-gelling extracts, respectively. The results of the bioluminescence assay are presented visually in **Figure 26**.

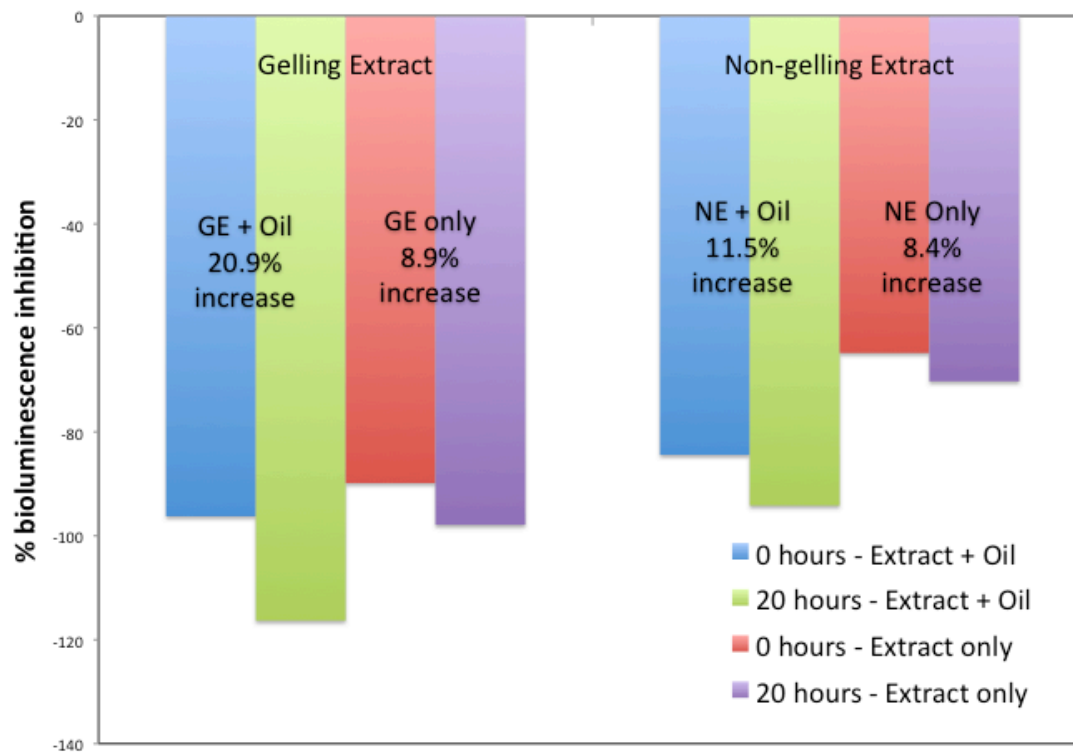


Figure 26: BLI results for gelling and non-gelling cactus mucilage extracts, n =3.

5 CONCLUSIONS AND IMPLICATIONS

Crude oil spilled in the open ocean during the Deepwater Horizon oil spill was subjected to many physical and chemical processes that ultimately affected its overall fate and toxicity. Corexit, and other dispersants designed for use in oil spill clean up, are designed to facilitate the biodegradation and eventual removal of toxic crude from the environment. However, research to this point has yielded conflicting evidence regarding both the toxicity and biodegradability of dispersants and dispersed oil. The results of the present study suggest sunlight may transform dispersed crude oil in such a way that it becomes more bioavailable to marine microorganisms and potentially facilitating biodegradation of toxic compounds.

Chemical components of dispersed crude oil degrade significantly after exposure to sunlight. Concentrations of hydrophobic petroleum hydrocarbons decreased more than 78% after exposure to 48 hours of sunlight with half-lives of these compounds estimated to be approximately 8.2 hours ($k' = 2.35 \times 10^{-5} \text{ s}^{-1}$). In this case, hypothetically speaking, if the surface of the ocean received 8 hours of intense sunlight a day, nearly half of the dispersed oil that reached the surface would be degraded within a day. Fluorescence from components extracted from dispersed oil samples decreased with half-lives on the order 9-10 hours. A decrease in fluorescence is indicative of molecular transformations of fluorescent compounds. Considering a hypothetical 8-hour day, the fluorescence of these components decreased by half in 1.2 days.

In an engineered UV system emitting at 254 nm, both direct and indirect photolysis contribute to the degradation of petroleum hydrocarbons in dispersed oil. The

rate constants for direct and sensitized photolysis via hydroxyl radicals ($k'_{\text{direct}} = 7.78 * 10^{-4} \text{ cm}^2 \text{ mJ}^{-1}$, $k'_{\text{indirect}} = 1.05 * 10^{-3} \text{ cm}^2 \text{ mJ}^{-1}$) were similar indicating the degradation of petroleum hydrocarbons is highly dependent on the scavengers present in the water. Natural seawater contains anionic and organic compounds that may scavenge reactive oxygen species and slow the degradation of crude oil components.

The transformation of crude oil components and amplified bioluminescence of *V. fischeri* indicate sunlight degradation may be a vital process in increasing bioavailability and removing toxic components following dispersant application to crude oil spills in the ocean. Bioluminescence of *V. fischeri* is inherently linked to its metabolism of compounds in the aqueous matrix. The BLI assay described in this study is designed in such a way that the bacterium is removed from its growth media and placed in a matrix that either suppresses or promotes activity, indicated by an increase or a decrease in bioluminescence, respectively. The BLI assay suggests that dispersed oil becomes less toxic as its exposure to sunlight increases. In fact, sunlight-degraded dispersed oil enhanced the activity of *V. fischeri* during the course of the tests demonstrating a possible increase in bioavailability of crude oil components. In contrast, sunlight exposure caused a toxic effect in oil only samples and did not affect the toxicity of dispersant only samples. These findings were supported by results from BLI assays of cactus mucilage-dispersed oil. Bioluminescence of *V. fischeri* was also enhanced after mucilage-dispersed oil samples were exposed to sunlight.

The BLI assay, as with any biological assay, does not come without its limitations. The BLI assay only evaluates the effects of potential toxicants on mechanisms of cellular respiration of *Vibrio fischeri*. Dispersed crude oil may exhibit

other mechanisms of toxicity not encompassed by the BLI assay. Additionally, it is possible that *V. fischeri* may not be sensitive to components of crude oil and the results of the assay are not representative of microbial toxicity in the ocean. While these limitations are a distinct possibility, *V. fischeri* is still commonly used to assess the toxicity of environmental contaminants including those associated with the Deepwater Horizon spill (Mearns et al., 2010; King et al., 2014; Paul, et al., 2013).

Crude oil released into the environment may have a detrimental effect on marine habitats; however, there are many natural processes that may reduce the concentrations of toxic compounds, thus minimizing the potential impacts. Results of this study indicate sunlight degradation is a vital first step in increasing bioavailability, particularly for dispersed crude oil, which supports data found elsewhere (Guo et al., 2013; King et al., 2013; Zhou et al., 2012; 2013). This may impact how dispersant is applied to oil spills in the future considering the large quantity applied to the plume below the surface of the ocean to prevent it from reaching the surface. Sunlight is a cheap and fast remediation tool that should be considered when designing dispersants and treating inevitable future spills.

REFERENCES

- Baelum, J., Borglin, S., Chakraborty, R., Fortney, J. L., Lamendella, R., Mason, O. U., ... Jansson, J. K. (2012). Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environmental microbiology*, 14(9), 2405–16. doi:10.1111/j.1462-2920.2012.02780.x
- Bolton, J. R., Linden, K. G., & Asce, M. (2003). Standardization of Methods for Fluence, UV Dose ... Determination in Bench-Scale UV Experiments, (March), 209–215.
- BP oil spill | Environment | The Guardian. (n.d.).
- Brooijmans, R. J. W., Pastink, M. I., & Siezen, R. J. (2009). Hydrocarbon-degrading bacteria: the oil-spill clean-up crew. *Microbial biotechnology*, 2(6), 587–94. doi:10.1111/j.1751-7915.2009.00151.x
- Bugden, J. B. C., Yeung, C. W., Kepkay, P. E., & Lee, K. (2008). Application of ultraviolet fluorometry and excitation-emission matrix spectroscopy (EEMS) to fingerprint oil and chemically dispersed oil in seawater. *Marine pollution bulletin*, 56(4), 677–85. doi:10.1016/j.marpolbul.2007.12.022
- Campo, P., Venosa, A. D., & Suidan, M. T. (2013). Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25 °C. *Environmental science & technology*, 47(4), 1960–7. doi:10.1021/es303881h
- Contributors, U. S. E. P. A. O. R. D., Hemmer, M. J., Barron, M. G., & Greene, R. M. (2010). Comparative Toxicity of Louisiana Sweet Crude Oil (LSC) and Chemically Dispersed LSC to Two Gulf of Mexico Aquatic Test Species U . S . Environmental Protection Agency Office of Research and Development, 1–13.
- D'Auria, M., Emanuele, L., Racioppi, R., & Velluzzi, V. (2009). Photochemical degradation of crude oil: Comparison between direct irradiation, photocatalysis, and photocatalysis on zeolite. *Journal of hazardous materials*, 164(1), 32–8. doi:10.1016/j.jhazmat.2008.07.111
- Dong, M. M., & Rosario-Ortiz, F. L. (2012). Photochemical formation of hydroxyl radical from effluent organic matter. *Environmental science & technology*, 46(7), 3788–94. doi:10.1021/es2043454
- George-Ares, A., & Clark, J. R. (2000). Aquatic toxicity of two Corexit® dispersants. *Chemosphere*, 40(8), 897–906. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0045653599004981>
- Glover, C. M., Mezyk, S. P., Linden, K. G., & Rosario-Ortiz, F. L. (2014). Photochemical degradation of Corexit components in ocean water. *Chemosphere*, 111, 596–602. doi:10.1016/j.chemosphere.2014.05.012
- Goodbody-gringley, G., Wetzel, D. L., Gillon, D., Pulster, E., Miller, A., & Ritchie, K. B. (2013). Toxicity of Deepwater Horizon Source Oil and the Chemical Dispersant , Corexit H 9500 , to Coral Larvae, 8(1). doi:10.1371/journal.pone.0045574
- Gulf Oil Spill : Pictures, Videos, Breaking News. (n.d.).
- Guo, L., & Lee, H. K. (2011). Low-density solvent-based solvent demulsification dispersive liquid-liquid microextraction for the fast determination of trace levels of sixteen priority polycyclic aromatic hydrocarbons in environmental water samples. *Journal of chromatography. A*, 1218(31), 5040–6. doi:10.1016/j.chroma.2011.05.069

- Hayworth, J. S., & Clement, T. P. (2012). Provenance of Corexit-related chemical constituents found in nearshore and inland Gulf Coast waters. *Marine pollution bulletin*, 64(10), 2005–14. doi:10.1016/j.marpolbul.2012.06.031
- Holmes-Smith, a. S., Uttamlal, M., McCormick, C., Hepburn, D. M., Graham, a., & Faichnie, D. (2012). Fingerprinting of crude oil using fluorescence spectroscopy. (W. W. Hou & R. Arnone, Eds.), 8372, 83720D–83720D–10. doi:10.1117/12.920604
- King, S. M., Leaf, P. a, Olson, A. C., Ray, P. Z., & Tarr, M. a. (2014). Photolytic and photocatalytic degradation of surface oil from the Deepwater Horizon spill. *Chemosphere*, 95, 415–22. doi:10.1016/j.chemosphere.2013.09.060
- Korak, J. a, Dotson, A. D., Summers, R. S., & Rosario-Ortiz, F. L. (2014). Critical analysis of commonly used fluorescence metrics to characterize dissolved organic matter. *Water research*, 49, 327–38. doi:10.1016/j.watres.2013.11.025
- Kover, S. C., Rosario-Ortiz, F. L., & Linden, K. G. (2014). Photochemical fate of solvent constituents of Corexit oil dispersants. *Water research*, 52, 101–11. doi:10.1016/j.watres.2013.12.013
- Long, S. M., & Holdway, D. A. (2002). Acute toxicity of crude and dispersed oil to Octopus pallidus, 36, 2769–2776.
- Lyman, J., Fleming, R. H. (1940). Composition of seawater. *Journal of Marine Research*, 3: 134–146.
- Mearns, A. J., Reish, D. J., Oshida, P. S., & Ginn, T. (2010). Effects of Pollution on Marine Organisms. *Water Environment Research*, 82(10), 2001–2046. doi:10.2175/106143010X12756668802175
- Mufson, S. (2012). BP settles criminal charges for \$4 billion in spill; supervisors indicted on manslaughter - The Wash. Washington Post. Retrieved from http://www.washingtonpost.com/business/economy/bp-to-pay-billions-in-gulf-oil-spill-settlement/2012/11/15/ba0b783a-2f2e-11e2-9f50-0308e1e75445_story.html
- Paul, J. H., Hollander, D., Coble, P., Daly, K. L., Murasko, S., English, D., ... Kovach, C. W. (2013). Toxicity and Mutagenicity of Gulf of Mexico Waters During and After the Deepwater Horizon Oil Spill.
- Prince, R. C., McFarlin, K. M., Butler, J. D., Febbo, E. J., Wang, F. C. Y., & Nedwed, T. J. (2013). The primary biodegradation of dispersed crude oil in the sea. *Chemosphere*, 90(2), 521–6. doi:10.1016/j.chemosphere.2012.08.020
- Questions and Answers | EPA Response to BP Spill in the Gulf of Mexico | US EPA. (n.d.).
- Rico-Martínez, R., Snell, T. W., & Shearer, T. L. (2013). Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A(®) to the *Brachionus plicatilis* species complex (Rotifera). *Environmental pollution (Barking, Essex : 1987)*, 173, 5–10. doi:10.1016/j.envpol.2012.09.024
- Ryder, A. G. (2005). ANALYSIS OF CRUDE PETROLEUM OILS USING FLUORESCENCE SPECTROSCOPY. In C. D. (Institute of F. and C. for F. S. Geddes & U. of M. S. of M. Lakowicz, Joseph R (Center for Fluorescence Spectroscopy (Eds.), *Reviews in Fluorescence* 2005 (pp. 169–198). Springer US. doi:10.1007/0-387-23690-2_8
- Shemer, H., & Linden, K. G. (2007). Aqueous photodegradation and toxicity of the polycyclic aromatic hydrocarbons fluorene, dibenzofuran, and dibenzothiophene. *Water research*, 41(4), 853–61. doi:10.1016/j.watres.2006.11.022
- Size and Percent Coverage of Deepwater Horizon:BP Oil Spill Closures : Southeast Regional Office. (n.d.).

- Team, N. R. (2011). Coordinator Report Deepwater Horizon, (September).
- Venosa, a D., & Holder, E. L. (2007). Biodegradability of dispersed crude oil at two different temperatures. *Marine pollution bulletin*, 54(5), 545–53. doi:10.1016/j.marpolbul.2006.12.013
- Zhou, Z., Liu, Z., & Guo, L. (2013). Chemical evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition. *Marine pollution bulletin*, 66(1-2), 164–75. doi:10.1016/j.marpolbul.2012.09.028

Appendix

Abbreviations

2-BE: 2-butoxyethanol

BLI: Bioluminescence inhibition

BP: British Petroleum, the company responsible for the Deepwater Horizon blowout.

cm: Centimeter

DGBE: Dipropylene glycol monobutyl ether

DLLE: Dispersive liquid-liquid extraction

DOM: dissolved organic matter

DOSS: dioctyl sodium sulfosuccinate

DWH: Deepwater Horizon

EEM: Excitation-emission matrix

EPA: Environmental Protection Agency

ETPH: Extractable Total Petroleum Hydrocarbons

g: gram

GC-FID: Gas chromatography – Flame ionization detector

GE: Gelling extract (refers to cactus mucilage)

kg: kilogram

L: liter

LP UV: low pressure ultraviolet

min: minute

mL: milliliter

NE: Non-gelling extract (refers to cactus mucilage)

nm: nanometer

OD₆₀₀: Optical density at 600 nm

ODR: Oil-to-dispersant ratio

PAH: polyaromatic hydrocarbon

pCBA: 4-para-chlorobenzoic acid

PG: Propylene glycol

ppm: parts per million

psi: pounds per square inch

RT: Retention time

RU: Raman Units

s: seconds

TPH: Total Petroleum Hydrocarbons

uL or μ L: microliter

UV: Ultra violet (refers to ultra violet light)

UV-Vis: Ultra violet, visible (refers to the ultra violet and visible light spectrum)

W: Watts

WAF: Water accommodated fraction