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### Publication Date

2013

Peer reviewed|Thesis/dissertation

**Oil Spill Remediation and Restoration:  
The Fate and Consequences of Oil in the Environment**

By

Thomas James Azwell

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Science, Policy & Management

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Garrison Sposito, Chair

Professor Keith Gilless

Professor David Zilberman

Fall 2013



## Abstract

### Oil Spill Remediation and Restoration: The Fate and Consequences of Oil in the Environment

by

Thomas James Azwell

Doctor of Philosophy  
in  
Environmental Science, Policy & Management

University of California, Berkeley

Professor Garrison Sposito, Chair

The dissertation is concerned with the effects of oil spills on the environment, using the 2010 Gulf of Mexico event, which motivated the selection of this research topic, as a case study. It examines the spill from both a social and biological assessment in which policy, technology, and economics direct oil spill response and remediation.

The dissertation is partly based on material collected during two years of fieldwork in Southern Louisiana. The Deepwater Horizon case study includes qualitative research grounded in a participatory action research (PAR) approach. The PAR strategy includes collective inquiry and experimentation through direct experience.

Although historically the effort to mitigate the effects of the Deepwater Horizon spill was the greatest cleanup response to an oil spill, the effort only affected 24% of the oil released in the Gulf. The fate of the remaining oil is unknown. Natural gas was not included in the spill discharge metric, nor will recovered oil (skimming and siphoning) be deducted from the fine that will be assessed on the responsible party, British Petroleum. Response strategies, such as the use of chemical dispersants and in-situ burning, did not remove oil, but instead contributed to the cumulative pollution in the environment. This case study revealed an opportunity to create legislation that motivates increased investment in technologies and response strategies that support the removal of the oil from the environment.

Trough the Deepwater Horizon case study, I also explored alternative spill response technologies and approaches to remediation and restoration. More than a dozen alternative technologies were evaluated and adopted during the 87-day oil spill event. The technologies evaluated included advancements for oil removal — skimming and shoreline cleanup. Furthermore, for the first time, an oiled marsh was set aside for the purpose of conducting applied oil remediation and restoration

research. Through a multi-institutional collaboration, we designed and implemented a restoration project on set-aside marsh in Louisiana. This project abandoned the use of cultivars and instead embraced genetically diverse, locally adapted plants for shoreline restoration. Included in the marsh project was a plant propagation innovation which utilized composted bagasse, a waste product of the Louisiana sugar cane industry, as a growth medium. The bagasse adds valuable organic material to the oil-impacted marsh and proved to be a viable propagation medium for smooth cordgrass (*Spartina alterniflora*) plants.

Additional soil remediation research, funded by the Chevron Corporation, investigated the use of vermiremediation for crude oil-impacted soils. Analysis of vermitea, the liquid extract from vermicompost, indicated the presence of biosurfactant producing hydrocarbonoclastic bacteria, allowing for the increased solubility of hydrophobic compounds adsorbed to soil. Additional research and field-scale experiments are required to optimize vermiremediation and demonstrate the potential for scaling and adoption.

My research supports the use of natural attenuation of oil-contaminated soil through the adoption of strategies which help to maintain the existing ecosystem. My research findings elucidate the critical limitations of current conventional oil spill response technologies and reveal the environmental tradeoffs that occur during response decision-making. The dissertation demonstrates the need for additional investment in technology innovation and for broader response strategies and preparation for future oil spills.

## Acknowledgements

The past six years have been an intense journey, both literally and figuratively. Along the road I have met people who in many ways helped shape my path in life. This is to express my appreciation to them.

First of all I want to thank my advisor Gary Sposito, committee members Keith Gillless, and David Zilberman. I am immensely grateful for their guidance, feedback, and willingness to support of my ambition to complete a doctoral degree at the University of California, Berkeley.

I want to thank the students and staff at the Department of Environmental Science, Policy, and Management (ESPM), especially Jeff Romm, Alastair Iles, Adrienne Hink, Sharon Bone, Alasdair Cohen, Jesse Williamson, and Josh Dimone. This is a special group of people whom without their support and friendship completing the degree requirements would not have been possible. It was their belief in me which gave me the confidence to succeed.

Finally, I want to thank my friends Barbara Nakakihara, Charlie Fahrmeier, Dana Ramirez, Amy Banas, and my family for their loving support. Without their love this journey would never been undertaken. To my Mother and her reminders that I'm "special", my sisters for keeping me grounded, my Father for his tough love and bootstrap philosophy for succeeding in life, and Lou Dee, who guided me to the end, it is to them this dissertation is dedicated.

To all, and to the many others I have failed to mention, a big thank you!

Thomas Azwell  
Berkeley, California  
Fall 2013

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# Chapter 1

## Introduction

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### 1.1 Deepwater Horizon Oil Spill

On April 20, 2010 the Deepwater Horizon oil rig exploded and leaked an estimated 4.9 million barrels (779 million liters) of crude oil 1,500 meters below the surface of the Gulf of México [1, 2]. This was the largest accidental oil spill in history, twenty times greater than the 1989 Exxon Valdez spill in Prince William Sound, Alaska [3, 4]. The event was reported to be, “the worst environmental disaster in U.S. history” [5], meaning worse than the Three Mile Island nuclear meltdown, the intensive industrial agriculture that lead to the Dust Bowl in the 1930s, or the 21,000 tons of industrial waste buried in Niagara Falls, New York in the 1940s (Love Canal) [6-8].

The purpose of my dissertation is to provide a comprehensive environmental impact assessment of the Deepwater Horizon oil spill, to summarize the lessons learned from the spill and associated cleanup efforts, and to present research focused on remediation of crude oil impacted soils. The information contained in my dissertation is intended to provide a framework for guiding future spill response, a better understanding of the inherent risks involved in oil exploration and production, and an introduction to advances in remediation strategies and technologies.

### 1.2 Oil Spill Response Technology

The US Coast Guard is tasked with leading the response effort for marine oil spills as described in the Federal Water Pollution Control Act [9, 10]. Oil spill response includes a suite of protocols carried out by various government agencies, oil spill contractors, trained volunteers, and employees of the responsible party. The objective is to contain, recover, and disperse the oil before it is lost to the environment [11]. Conventional oil spill response technologies comprise a set of universally adopted tools; each tool has a distinct environmental and economic tradeoff [12, 13].

There have been great technological advancements made in oil exploration and extraction, including, for instance, remotely operated vehicles, ultra-deep water platforms (more than 3,000 meters), and four-dimensional seismic imaging, used to locate potential deep sea reservoirs [14, 15]. However, these technological achievements are limited to removing large volumes of oil from beneath the surface of the ground, not from the surface of soil or water.

The objective of the case study presented in Chapter 2 is to examine the suite of conventional technologies used during the Deepwater Horizon incident for oil removal from the surface of the water, before it reaches sensitive shoreline ecosystems [16, 17]. It is important to relate the decision-making process carried out by the Incident Command system and the environmental tradeoffs related to response tools [12]. This information should provide a more robust guide to future spill response, as well as a better understanding of the environmental health risks involved in oil exploration and production. The review serves as the start of a larger conversation about environmental regulation and oil spill fine assessment, and exposes the need for investment in environmentally sensitive and effective oil removal and cleanup technologies [12, 17, 18].

### **1.3 Remediation and Restoration**

The National Contingency Plan (NCP) is a procedural guide for a national response effort [19]. The plan provides an effective management strategy and establishes the hierarchy of responders, coordination of area contingency plans, system for reporting accidents, and a funding mechanism and guide for the deployment of response technologies [19, 20]. The immediate oil spill response or “remediation” phase is primarily focused on preventing oil from entering the water column, reaching the benthic sediments or a shoreline ecosystem [11]. However, the ability of agencies and contractors to prevent oil from reaching shorelines and sediments is limited by the efficacy of the available spill response technology [21-24].

The volume of oil that is unaffected by the primary response effort, 2.9 million barrels in the case of the Deepwater Horizon spill, determines the level of degradation and the need for environmental restoration [2, 25-27]. Marsh macrophytes are important to the ecological health of the Gulf of México coastline [28, 29]. Oil that was not recovered offshore near the incident site eventually found its way to these sensitive marsh ecosystems which now require restoration [30].

Chapter 3 examines the environmental damage to the Louisiana coastal marshes that arose from the limitations of conventional oil spill response technology, as

described in the previous chapter [11]. The objective is to evaluate the federal government's response plan for mitigating environmental damage to the Gulf coast [19, 31]. This response plan also describes a process for evaluating and adopting alternative technologies during an incident, which was important to the Deepwater Horizon response effort [17, 32, 33]. Additionally, the objective is to examine strategies that were tested for remediating and restoring impacted shorelines [25, 30], including, for instance, my collaborative remediation research conducted on a "set aside" marsh in one of the most heavily oiled regions in the Gulf of México, Barataria Bay [34, 35]. The goal is to identify steps that could be taken to promote ecosystem recovery by linking shoreline remediation with habitat restoration, placing emphasis on local sourcing and novel approaches that reduce operational trade-offs and maximize efficiencies.

## **1.4 Bioremediation of Crude Oil in Soil**

The impact of oil pollution depends on the levels of the constituent pollutants, their toxicity, and their distribution, which influence the retention time of the various oil components in sediments (3). The toxicity of the contamination is higher when heavy metals are present and the task of removing petroleum-derived organic compounds becomes both more difficult and more complex (4, 5). Nature has the capacity to remediate low levels of contamination [36]. However, the fate of the oil is often to persist in the environment, especially when it adheres to soil and sediments. The longer chain hydrocarbons are more viscous and will bind to the soil particles, thus reducing the surface area available for bioremediation. It is best to utilize a remediation approach that models natural attenuation to help minimize disturbance and promote habitat recovery and restoration [37].

Chapter 4 investigates natural attenuation and the use of bioremediation strategies for polycyclic aromatic hydrocarbons (PAHs), which remain in the environment after an oil spill [38]. The objective of the chapter is to present data from an ongoing research project for developing tools for in-situ remediation of vadose zone weathered crude oil soil. The research evaluates biosurfactants produced by microorganisms, which increase the surface area and bioavailability of hydrophobic water-insoluble substrates, such as petroleum hydrocarbons [39]. The research looks at the limits of tolerance of earthworms to PAHs, evaluates the bioaccumulation of PAHs in earthworm tissues, and examines the use of thermophilic compost, hydrocarbon degrading microbes, and earthworms as a remediation strategy [40, 41].

It is important to continue research and development into strategies that support complete remediation of PAHs (especially the persistent long carbon-chain or

“heavy” fractions [42-45]. As the Deepwater Horizon case study discussed, a majority of oil from oil spills is lost to the environment despite the response effort [17, 46]. For example, oil recovery, chemical dispersion, and burning accounted for only 24 % of the 4.1 million barrels of crude oil that were effectively discharged into the Gulf of México [26]. The large amount of oil from the Deepwater Horizon oil spill that was unaccounted for may eventually bind to vegetation, sediment, and soil particles. Furthermore, there are frequent spill reported during the normal upstream processing of crude oil—extraction, transportation, and refinement—where oil can cause permanent damage when it is lost to the environment [47, 48].

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# Chapter 2

## Deepwater Horizon Oil Spill Remediation: Limitations and Environmental Tradeoffs of Spill Response Technology

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### 2.1 Introduction

The Deepwater Horizon oilrig exploded and began leaking 1,500 m below the surface of the Gulf of México on April 20, 2010. The Department of the Interior subsequently established a Flow Rate Technical Group, which estimated the leak initially produced 62,000 barrels of oil a day and eased to 53,000 barrels a day as the reservoir gradually lost some of its initial pressure [1]. In total, they concluded that 4.9 million barrels (779 million liters) of oil had discharged from the well before it was capped and sealed July 15, 2010 resulting in the worst accidental marine oil spill on record [2]. An estimated 205 million gallons of crude oil and 260,000-520,000 tons of methane (the energy equivalent of 80-155 million gallons of crude oil) were released into the Gulf of México over the following 87 days [3]. More than 40,000 responders aided control efforts over the course of the 89-day continuous discharge and deployed cleanup response technologies, which included containment and absorbent booms to slow the spread of the oil, in-situ burning to combust the oil on the water's surface, chemical dispersant applied at the surface and subsea to dilute the oil into the water column, and oil skimmers to contain and remove the oil from the environment [4]. Addressing both surface and subsurface conditions posed unanticipated challenges to governmental responses dictated by federal legislation shaped by traditional surface spills [5]. Response efforts not only identified major gaps in baseline knowledge of vulnerable ecosystems, but also demonstrated that advances in deep water drilling have far outpaced advances in spill containment and shoreline remediation [5].

The purpose of this chapter is to present an overview of oil spill response decision-making and both conventional<sup>1</sup> and alternative technologies deployed during the Deepwater Horizon event, as well as summarize the lessons learned from the spill and the challenges faced during the immense cleanup effort. This information should provide a more robust guide to future spill response, as well as a better understanding of the environmental health risks involved in oil exploration and production. The chapter also details the changing nature of oil in the environment specific to the Deepwater Horizon spill; outlines the tradeoffs of response tools and

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<sup>1</sup> Conventional technologies represent those technologies which have been widely adopted and deployed by the greater spill response community.

decisions made by Incident Command; and serves as the start of a larger conversation regarding regulation, fine assessment, and the need for more investment in developing environmentally sound technologies (ESTs)<sup>2</sup>.

## 2.2 Incident Command System

The National Oil and Hazardous Substances Pollution Contingency Plan (NCP) serves as the federal government’s guide for strategizing a response to oil spills in federal waters [6]. The United States Coast Guard (USCG) was charged with overseeing the Deepwater Horizon oil spill response effort in accordance with the National Contingency Plan. Facilitating a response to a “spill of significance” requires an organizational management system for guiding the response. The Incident Command System (ICS) provides a framework necessary for coordinating the effort of the government agency response organizations [7, 8]. The ICS was originally developed through a cooperative effort among federal, state, and local governmental agencies to combat a series of massive wildfires in California during the 1970s. The system helped to manage the complexities associated with authority as the fire moved across jurisdictional borders [7].

***“ICS is flexible and should be viewed as a response tool, not a response rule”  
- U.S. National Response Team [8]***

The Federal responders established command posts, lead by the USCG, allowing for shared facilities and improved communication. A Unified Area Command (UAC) center has the main objective of managing the daily deployment of personnel and spill response tools in an effort to mitigate the impacts of oil to the local environment [8]. Three command centers were designated during the Deepwater Horizon incident due to the large geographical magnitude of the disaster—Houma, Louisiana; Mobile, Alabama; Miami, Florida; Houston and Galveston, Texas [9]. Traditionally, the set of “tools” includes oil spill cleanup agents, such as oil-water surface skimming devices, chemical dispersants, biological agents, in-situ burning, and containment and absorbent booms [10]. The Gulf oil spill response employed two main categories of technologies—oil dispersion and oil recovery. The latter, which includes absorbent booms, skimmers, and oil-water separators, could be considered environmentally preferable to oil dispersion because recovery tools function by removing oil from the environment—mitigating long-term environmental exposure [11]. In contrast, chemical dispersion does not remove oil from the water but changes its distribution from the water surface to the water column.

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<sup>2</sup> Technologies that have the potential for significantly improved environmental performance relative to other technologies.

The incident command structure includes five essential response operational functions: Command, Operations, Planning, Logistics and Finance and Administration [9]. This division of responsibility helps to create an organizational framework for successful disaster response. The agency organizational structure in spill response, in hierarchical order by authority, is the National Response Team (NRT), Regional Response Team (RRT), and the Area Committee (AC)<sup>3</sup>.

The Deepwater Horizon oil spill presented a unique challenge for responding agencies due to the scale, both the volume of pollution and its duration, and the need to coordinate efforts of local government, state government, including Parishes<sup>4</sup>, the federal government and the Response Party (RP), British Petroleum p.l.c. (BP).

### 2.3 Crude Oil Behavior and Weathering



**Figure 2.1:** Weathered crude oil – Gulf of México. Image courtesy of Andy Nyman, Louisiana State University.

When oil enters the environment from spills, ruptures, or blowouts it moves by three modes—horizontal transport of the surface slick, and both vertical and horizontal subsurface transport of dispersed oil [12, 13]. Turbulence near the surface can drive fresh oil droplets into the water column, but droplets of diameter greater than 60-80  $\mu\text{m}$  will generally resurface [14]. Therefore, as the slick's viscosity increases with evaporation and emulsification, turbulence will drive fewer droplets into the water column.

When oil remains in the environment for an extended period of time, it undergoes a continuous series of compositional changes that are the result of a process known as weathering (Figure 2.1) [15-17]. During this physico-chemical process, lighter oil components photo-oxidize and enter the atmosphere, while heavier oil components typically mix with water to form a viscous emulsion that is resistant to rapid weathering changes. Thus, it is slower to degrade, more persistent in the environment than non-emulsified oil, and more likely to enter the water column [18]. The oil emulsion's viscous character poses a threat to marine vegetation through covering and smothering surfaces with which it comes in contact. If the oil emulsion enters the water column and reaches the benthic zone, it may cause permanent damage to root systems, inhibiting the plant's ability to regenerate [19-21]. Emulsified oil cannot effectively be recovered by skimming technologies or

<sup>3</sup> Federal website for the Deepwater BP oil spill response and recovery, <http://www.restorethegulf.gov>

<sup>4</sup> An administrative division in Louisiana, equivalent to a county

absorbent booms, chemically dispersed, or burned. Thus, recovery efforts should be prioritized prior to significant emulsion of oil [22, 23].

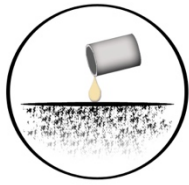
In addition to emulsification, oil in the Gulf also was dispersed through natural physical processes, which include weathering, evaporation, oxidation, biodegradation, and emulsification, as well as through interactions with chemical compounds [18]. The net effect of both natural and chemical dispersion was that much of the oil was transformed into tiny droplets with diameters less than 100  $\mu\text{m}$  [2]. Such droplets face significant flow resistance from the water column in their effort to rise to the surface [14]. They are trapped in the deep Gulf environment, where they may be degraded by bacteria, and are more likely to interact with marine life [3]. This dispersed oil is diluted as it moves away from the wellhead, some of its components dissolve into the water column and are available for fairly rapid biodegradation, while more refractory components are only slowly degraded by microorganisms [19]. Because the concentration of the dispersed oil was far lower than the concentration of dissolved oxygen in deep Gulf waters, oxygen depletion to levels that could harm marine fauna were not observed based on results from the Dispersant Application Plan monitoring data [24].

## **2.4 Conventional Oil Spill Response and Technology**

The chemical and physical composition of oil, as well as the hydrology of the ocean and climate conditions, determine the behavior and outcomes of offshore oil spills. The same factors influence the effectiveness of methods for removing oil from the ocean surface [25, 26]. Few feasible options are available for offshore treatment, however, once oil undergoes weathering through evaporation, dissolution, biodegradation, and photooxidation [17].

The Gulf oil spill response employed two main categories of technologies—oil dispersion and oil recovery [27]. The latter—which includes absorbent booms, skimmers, and oil-water separators—is environmentally preferable to dispersion because oil recovery tools work on the premise of completely removing the pollution from the environment—mitigating environmental exposure. “Environmentally preferable” means the response decision which has the greatest net environmental benefit [28]. In contrast, oil dispersion does not remove oil from the water but instead changes its distribution, for example with the use of chemical dispersants, from the water surface to the water column. In-situ burning, another common example of dispersion, removes most of the oil from the surface—changing liquid crude oil to a gas—leaving dense smoke in the atmosphere and a residue which may or may not be possible to collect from the water surface [29, 30].

## 2.4.1 Chemical Dispersants and their Role in Oil Spill Response



**“The real key to effective decision-making regarding dispersant use is a fuller understanding of the implications of alternative outcomes in the decision-making process” [31]**

Dispersants “are chemical agents that emulsify, disperse, or solubilize oil into the water column or promote the surface spreading of oil slicks to facilitate dispersal of the oil into the water column” [32]. They move the oil from the water surface to the water column by changing the surface tension and cohesive capacity of the oil, thus forming smaller droplets [33]. Their use does not reduce the total volume of oil in the environment, but rather changes its distribution in a body of water [34]. The use of chemical dispersants can be described in terms of a risk-based paradigm in which tradeoffs between environmental benefits and harms must be considered.

Spill-response decision makers recommend deploying dispersants only when an oil slick is an imminent threat to a sensitive shoreline ecosystem [33, 35]. Offshore spills produce an oil slick, which moves as a function of environmental factors including water and air velocities. If an oil slick is likely to make landfall, chemical dispersants may be applied to disaggregate the oil, allowing it to enter the water column. Due to limitations of conventional oil recovery technology during a spill of significant volume, chemical dispersants have become the de facto primary response technology [36].

Historically, chemical dispersant use dates back to 1966 when it was applied offshore during an oil tanker spill near Germany [37]. The subsequent decision to use chemical dispersants as a spill response tool became the status quo despite some evidence of their potential net harm. For example, in 1967 an oil tanker carrying 144.6 million liters (approximately 900,000 barrels) grounded on the Seven Stones Reef between Lands End and the Scilly Isles in the United Kingdom [35]. The resulting oil slick covered 700 square kilometers of sea. To combat the oil, approximately 1.6 million liters of dispersant were used in addition to the application of aviation fuel near the disabled vessel for the purpose of facilitating in-situ burning [38]. As a result, a dispersant-oil emulsion formed which persisted in the environment [39]. The dispersed oil affected 150 km of Cornish coastline, bringing into question the environmental tradeoffs in the decision. Because of the potential environmental harm resulting from adding pollutants to the water, such as a petroleum distillate, decision-makers must weigh the risk of choosing to disperse versus to skim and remove the oil [28].

During the Deepwater Horizon event, dispersants were used both on the surface of the ocean, spraying by plane (“carpet bombing”, see Figure 2.2) or by boat, and at 1,500 meters by direct injection into the flowing oil [24]. The decision to use dispersant in subsea water was an effort to reduce volatile organic compounds



**Figure 2.2:** Aerial spraying of chemical dispersant, a method referred to as "carpet bombing". Image courtesy of Stephen Lehmann, USCG.

(VOCs) at the surface, near the incident site where several vessels were working on capping the wellhead and recovering oil from an insertion tube [36]. Although the subsea use of dispersants is an approved method by the Environmental Protection Agency, it was the first time that it had been applied at depth; thus no trials exist to help determine efficacy and fate of the dispersant under these conditions—low temperatures combined with extreme pressure (40 °F/4.4 °C, 2,500 PSI) [2, 27]. In the case of the Deepwater Horizon event, the Environmental Protection Agency and Coast Guard directive required British Petroleum to measure dissolved oxygen and to

perform toxicity tests (LC<sub>50</sub>) on rotifers<sup>5</sup> to monitor the environmental impact [40]. The LC<sub>50</sub>, or a lethal concentration 50 %, is a result of a laboratory test of the concentration of values of chemical in water that kills 50 % of the test animals after a given time [41]. Normal values of dissolved oxygen concentration in the Gulf of México water at depth are approximately 4 mg/l; the directive was an effort to manage decreases below 2 mg/l, a level determined to be a measurable risk to aquatic life [42, 43].

The rationale for approving the discharge of chemical dispersants during oil spills is that dispersed oil will come to shore more dilute, with a less acute toxicity [12, 31, 33, 44]. However, some research suggests that dispersed oil is more immediately toxic to marine organisms because chemical dispersion increases bioavailability, meaning it is more readily absorbed [44-46]. The environmental tradeoffs regarding the use of chemical dispersants, summarized in **Table 2.1**, necessitate careful consideration prior to their application during oil spill response. The benefits include the potential for more rapid biodegradation of oil due to increased surface area, which results in better protection of shorelines. The harms include greater exposure of oil to subsurface marine life, no possibility of oil recovery and, when applied beneath the water surface, larger oil plumes of uncertain fate and environmental impact [44].

**Table 2.1:** The Tradeoffs of Chemical Dispersant Use [31]

<b>Benefit</b>	<b>Features</b>	<b>Associated Harm</b>
protection of shoreline	dispersion into the water column dilution of oil	greater exposure to the open ocean ecosystem oil cannot be recovered

<sup>5</sup> Rotifers are zooplankton found in both fresh and marine water environments (<http://www.ucmp.berkeley.edu/phyla/rotifera/rotifera.html>). The EPA directive called for the use of Rototox, a commercially-available rotifer test that can be performed on the deck of a ship ([www.epa.gov/bpspill/dispersants-qanda.html#q02](http://www.epa.gov/bpspill/dispersants-qanda.html#q02)).



Potential for increased biodegradation rate	increased surface area of oil to water dilution of oil	greater bioavailability oil cannot be recovered
more efficient oil dispersion with subsurface application	Injection of dispersant into the oil flow at the wellhead (source)	More subsurface oil in addition to that physically-dispersed

The use of chemical dispersants in marine oil cleanup is often supported by the benefits of an increased oil biodegradation rate. Their application is considered to have “net environmental benefit” by increasing the surface area available for microbial degradation from breaking an oil slick into small droplets, supporting the dilution of oil to concentrations of lesser harm to biota, and minimizing nutrient limitations, also by dilution [34]. In general, microbial degradation may be enhanced by increased surface area available for microbial attachment, and inhibited by dispersant toxicity or its substitution for oil as the carbon source. Many small droplets of oil have a larger surface area than one large slick, meaning potential increased access for microorganisms capable of degrading hydrocarbons [47].

An increase in microbial degradation was verified in a bench-scale study of chemically-dispersed Alaska North Slope crude oil artificially weathered to simulate naturally-occurring losses by evaporation [48]. The study found that chemically-dispersed oil resulted in an increase in the population of hydrocarbon-degrading bacteria as compared to control samples of naturally-dispersed oil. Further increasing the rate of biodegradation, the dispersed oil droplets were colonized by hydrocarbon degraders more quickly than undispersed oil. A correlation between microbial colonization and temperature, in which higher temperatures resulted in more rapid colonization, was also found. One can conclude that the warm waters of the Gulf of México would support more rapid oil biodegradation when compared to cooler waters under otherwise identical conditions. Another bench-scale study testing conditions non-conducive to dilution, like those that may be found in a wetland, the chemical dispersant application did not increase microbial degradation of the oil [49]. Although an increase in surface area generally results in an increase in degradation rates, there does not appear to be a general consensus in the science community on whether a chemical dispersant application increases biodegradation rate of crude oil [50-54]. The biodegradation may be limited by factors other than the total oil surface area, such as chemical dispersant toxicity, surfactant interference with the microbial attachment mechanism, or dispersant substitution for crude oil hydrocarbons as a microbial carbon source [34, 50].

A consequence of subsurface chemical dispersant use is the persistence of oil in deep waters. Fluorometry measurements in the Gulf of México suggest the presence of dispersed oil between approximately 1000 and 1300 meters depth and within a

10 km radius of the MC252 wellhead [43]. Oil both naturally- and chemically-dispersed below the water surface will rise at a rate dependent on droplet size, with small droplets of less than about 100  $\mu\text{m}$  in diameter capable of remaining suspended in the water column rather than rising to the surface [14]. With the addition of 6.8 million liters of Corexit 9500 and 9527A dispersant, surface oil penetrated to about a 6-m depth in the water column, creating oil plumes [55]. Subsea dispersant use near the wellhead, as well as methane dissolution, also contributed to plume formation [56]. Very little research data exist on the fate or ecological impacts of oil plumes increased by subsurface dispersant application. Low dissolved oxygen may limit microbial degradation of hydrocarbons, resulting in persistence of dispersed oil plumes at depth [55].

***“Approximately 1.84 million gallons of total dispersant have been applied—1.07 million on the surface and 771,000 subsea.”***

***- Restore the Gulf [24]***

The total volume of chemical dispersant used in the Gulf of México exceeded 6.8 million liters, with nearly 42 % applied near the leaking wellhead as a subsurface application, and 58 % at the ocean surface [57]. The peer-reviewed oil mass balance produced by the Federal Interagency Solutions Group indicates that 16 % of the total volume of oil, nearly 125 million liters, was chemically dispersed and that 13 % of the oil, over 94 million liters, was naturally dispersed [2]. Thus, the ratio of dispersed oil to chemical dispersant by volume, according to their estimate, is about 18:1 and chemically dispersed oil accounted for more than half of total dispersed oil. This assumes that subsea use of dispersant was highly effective. The Environmental Protection Agency approved the application of dispersants subsea, however because this was the first such application, there were no trials related to efficacy or dispersant fate available for reference [58].

In response to uncertainties regarding the use of dispersants during the Deepwater Horizon event, the Environmental Protection Agency completed two toxicity test on chemical dispersants [59]. These tests examined the acute toxicities of various approved (National Contingency Plan) chemical dispersants via  $\text{LC}_{50}$  measurements, as well as the toxicity of chemically-dispersed oil as compared to non-chemically dispersed oil to the mysid shrimp, *Americamysis bahia*, and the inland silverside fish, *Menidia beryllina*. The findings are summarized in

**Table 2.2** [60]. The acronyms WAF and CE-WAF stand for water accommodated fraction and chemically enhanced water accommodated fraction, respectively. These are prepared water samples containing oil dispersed either with/without the use of chemical dispersant. As the  $\text{LC}_{50}$  increases, toxicity decreases, because more of the substance is required to cause equivalent mortality in the test population.

**Table 2.2:** Toxicity of Corexit 9500A in 2010 Environmental Protection Agency Studies [59]

Test organism	LC <sub>50</sub> (mg/kg)			Toxicity Classification		
	Corexit 9500A	CE-WAF	WAF	Corexit 9500A	CE-WAF	WAF
Mysid shrimp	42	5.4	2.7	Slightly toxic	Moderately toxic	Moderately toxic
Inland Silverside	130	7.6	3.5	Practically non-toxic	Moderately toxic	Moderately toxic

From the measurements shown in

**Table 2.2**, it can be concluded that Louisiana Sweet Crude (LSC) is no more toxic to the mysid shrimp and inland silverside fish when chemically dispersed than when dispersed non-chemically [44]. Both forms of dispersed oil show moderate toxicity, while Corexit 9500A alone is either slightly toxic or practically non-toxic to these species. The manufacturer of the Corexit line of dispersants reports an LC<sub>50</sub> of 25.2 parts per million (mg/kg) for the inland silverside fish [44]. This lower value would be classified as moderately toxic, as opposed to slightly toxic, based on the EPA's classification. The variability in toxicity under controlled laboratory conditions suggests that there would also be variability in toxicity when chemical dispersants are applied in the environment [54]. In addition, the trends found by these Environmental Protection Agency studies may not hold for all chemical dispersant and crude oil types. In a previous study on three freshwater species using Corexit 9500A, chemically-dispersed oil was found to be more toxic than oil dispersed non-chemically [45].

An LC<sub>50</sub> analysis only addresses acute toxicity, but does not consider the potential sub-lethal long term impacts to organisms, such as reproduction, endocrine disruption, immunity, bioaccumulation and neurologic effects [44, 61]. There are additional methods for measuring potential toxicity of a substance in the environment. For example, by using cell lines or tissues of local species of aquatic organisms in vitro, tests can be designed to provide a way to rapidly screen compounds for potential endpoints of interest. This method allows us to measure cellular responses in toxicity pathway assays using high-throughput tests [62]. Assays can then be run to measure the cytotoxic action of the chemical pollutant. Toxicity can be assessed by a number of measures, such as cell death, viability and functionality, morphology, energy metabolism or changes to the rate of cell growth [63]. Other measures include biodegradability or persistence of the substance in the environment and bioaccumulation or the bio-concentration factor (BCF). The bio-concentration factor is defined as the concentration of a particular chemical, in this case oil dispersant, in a tissue as compared to the concentration of the chemical in

water [64]. It is important to employ a robust measure of toxicity when determining the potential long-term effects on an aquatic ecosystem.

The Marine Well Containment Company is an oil industry established nonprofit entity tasked with operating and maintaining underwater spills<sup>6</sup>. In April 2012 the Marine Well Containment Company funded the manufacturing of a Subsea Fluid Dispersant System to respond to underwater spills. This system will be designed to be autonomously based so that it can be controlled remotely to inject chemical dispersant into an oil leak near the wellhead [65]. The net environmental benefits of using chemical dispersants as a tool for combating oil spills necessitate careful consideration prior to their application. The benefits can include more rapid biodegradation of oil and subsequent protection of shorelines by removing the oil from the surface, thus preventing it from reaching the shoreline in a concentrated state. The harms include greater exposure of oil to subsurface marine life, no possibility of oil recovery in the dispersed form, and when applied beneath the water surface, formation of oil plumes which have an uncertain fate and environmental impact. It is important to conduct more research in an effort to understand better the efficacy and fate of chemical dispersants at depth, including low oxygen and low-temperature environments.

#### 2.4.2 In-Situ Burning of Crude Oil



**“(In-situ burning) is a response technique to be employed when an oil slick is virtually uncontrolled with the potential to spread and contaminate additional areas” [66].**

Previous oil spills of significance, such as the Exxon Valdez spill off the Alaskan coast, exposed the challenge of quickly transporting conventional mechanical recovery technology to the incident site. In-situ burning, however, requires limited equipment—a boat, fire-resistant boom and an ignition element (“In-situ burning”, see Figure 2.3) [67, 68]. In-situ burning, igniting the oil and allowing it to burn off, is considered by the industry as an effective method for quickly removing oil from the water [69-71]. An oil slick must first be held in place using a fire-resistant containment boom. There must be enough oil to ignite and to maintain combustion for an extended period of time. Sea and wind conditions must also be favorable in order for burning to be considered a viable option [69]. If all these criteria are met, then it is possible to burn oil on the surface of the water [68, 70].

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<sup>6</sup> Company website, <http://www.marinewellcontainment.com>



**Figure 2.3:** In-situ burning of oil during the Deepwater Horizon spill. Image courtesy of MCS Justin Stumberg, U.S. Navy.

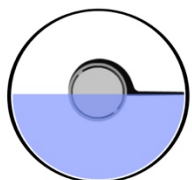
According to the oil mass balance calculation produced by the Department of the Interior, an estimated 5 % of the 4.1 million barrels<sup>7</sup> of oil that entered the Gulf of México in the Deepwater Horizon spill was burned [2, 72]. The combustion of crude oil forms a mixture of compounds in solid, liquid, and gaseous phases. The environmental impacts of in-situ burning are assessed by: the fraction of an oil layer that can be burned, the quantity of smoke, and the concentrations of 18 polycyclic aromatic hydrocarbons (PAHs) in the smoke, crude oil, and burn residue [30]. The minor components released, including particulate matter (PM), carbon monoxide (CO), sulfur dioxide (SO<sub>2</sub>), and nitrogen oxides (NO<sub>x</sub>), can have the greatest direct impact on human health. Volatile organic compounds (VOCs), which evaporate without ignition soon after reaching the surface, also are harmful if inhaled [73, 74]. The Environmental Protection Agency considers benzene, toluene, ethylbenzene, and xylene as the “key toxic VOCs” [75]. It is estimated that the 410 separate burns of approximately 245,000 barrels of oil released 635,000 to 2.1 million kilograms of black carbon (soot) into the atmosphere during the 3-month event [76]. This is equivalent to the amount of black carbon emissions normally released by all ships that travel the Gulf of México during the same 9-week period [30].

Dispersal of an airborne toxicant plume is controlled by local environmental factors, primarily wind speed and direction [77]. In previous oil spills where air quality was monitored following burning, concentrations of toxic gases fell to background levels outside approximately three kilometers from the burn [74]. If such dispersal is a general trend and burning takes place more than three kilometers offshore, harm to the general public – with respect to the aforementioned gases – will not be greatly increased by in-situ burning. Response workers near the burn, however, will be exposed to greater risk, necessitating the use of onboard air monitoring technologies [78]. Burning presents an occupational hazard to response personnel and the greater community due to the release of harmful gases and particulate matter into the atmosphere. The burning of oil on the water surface also represents lost opportunities in terms of oil recovery and the subsequent energy production by incineration. This information is useful in determining the net environmental benefits related to decisions in selecting appropriate spill response technologies [28].

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<sup>7</sup> 800,000 barrels of oil were "captured" from the wellhead through the use of an insertion tube and therefore did not enter the water.

### 2.4.3 Water Surface Oil Skimming Technology



**“The recovery element of a skimmer diverts or skims the oil from the sea surface, where it flows to the inlet side of a pumping system for transfer to storage” [79]**

Oil skimming technology is based primarily on the premise that oil is less dense than water and thus reaches the sea surface. Crude and other oils contain enough “light” fractions of hydrocarbons that the oil has a lower specific gravity than water and will remain on the surface for a period of time [80]. While the oil remains on the surface it can be skimmed and temporarily contained in storage tanks onboard a vessel, then transported and offloaded on shore.

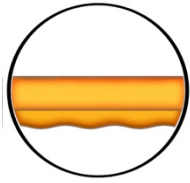
The skimmers move the surface water toward a containment system, which then transports the top layer of the oil-water mixture into a storage tank. Skimming efficacy is measured both by oil recovery rate, in barrels per hour (bbl/hr), and an efficiency factor, or oil-to-water ratio [79-81]. A typical weir skimmer will average about 600 bbl/hr with an efficiency of no more than 30 % oil-to-water [81]. During the DWH event, conventional skimming efficiencies were less than 30 % oil to water— a figure not uncommon to offshore oil response [27]. The X-Prize Foundation<sup>8</sup> recently hosted an oil spill cleanup challenge, sponsored by Shell Oil, which set a new standard for oil spill recovery. The winning skimmer performed at three times the industry’s current best recovery rate, achieving 6,671 barrels per hour recovery at 89.5 % oil-to-water efficiency; eleven times the typical weir skimmer average. The “grooved” disc design of the skimmer, which relies on capillary action, was created by a former University of California at Santa Barbara graduate student who is currently employed by Shell Oil<sup>9</sup>. The crude oil on the surface of the water is dynamic and changes as it loses its lighter fractions of hydrocarbons due to weathering [16, 17]. The oil recovery rate of the skimmer therefore decreases as the oil weathers and the viscosity increases [18, 79].

Boom is often paired with other technologies to remove oil from the ocean surface. Oil that is contained in rigid boom can be skimmed from the ocean surface into tanks onboard a vessel and transported to shore. Conventional skimmers move the surface water toward a recovery system that transfers surface and near-surface layers of oil-water mixtures into a storage tank. Conventional skimming can prove ineffective under adverse weather conditions that complicate containment and that promote subsurface mixing. The availability of equipment and personnel costs are two other major limiting factors to skimming oil from the water.

<sup>8</sup> Organization website, <http://www.iprizecleanoceans.org>

<sup>9</sup> <http://convergence.ucsb.edu/article/ivory-tower>

## 2.4.4 Oil Containment Boom Technology



During the Deepwater Horizon BP oil spill, response crews deployed more than two million feet of containment boom in the Gulf of México. Containment boom provided temporary protection to shoreline ecosystems. [27]

A spill containment boom is designed to prevent surface oil and debris temporarily from moving toward a shoreline. Its use during the Deepwater Horizon event was described as “protective booming” of priority areas, such as sensitive shoreline ecosystems (Figure 2.4) [27]. By definition, containment boom serves as a temporary barrier to oil on the surface, concentrating the oil for collection<sup>10</sup>. This bright-orange, vinyl-coated polyester rigid boom drafts slightly below the water surface, usually with a 30 cm flat piece of material attached, known as a skirt. The skirt helps hold oil in place, as it moves below the surface of the water, during strong tidal action<sup>11</sup>. After the three-month response to the Deepwater Horizon spill, British Petroleum reported a total of 1.3 million meters of containment boom was deployed to serve as a protective barrier for intertidal marshes, a device for holding oil in place for skimming, and a fire-resistant boom for the purpose of in-situ burning of the oil [27].



**Figure 2.4:** Oil containment boom deployed as a *temporary* protective barrier. Image courtesy of Andy Nyman, Louisiana State University.

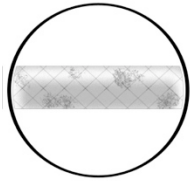
***“Failure of a boom to contain oil due to excessive winds, waves or currents, or improper deployment. Boom failure may be manifested in oil under-flow, oil splash-over or structural breakage.” [82]***

This oil spill response tool provided temporary protection of shoreline ecosystems, but due to the widespread oiling it was anchored and left in place for extended periods of time [4]. Containment boom is secured to the sea floor using Danforth anchors, but is subject to tidal forces and wave action, so eventually anchors will fail as will the boom’s ability to hold the oil for an extended period of time [27]. The containment boom was often paired with sorbent boom, as a secondary measure of defense, by the use of wire cable and carabineer clips. Due to changes in the oils viscosity from weathering or high tidal action, the oil eventually moves past the boom and to nearby shorelines [24].

<sup>10</sup> <http://www.incidentnews.gov/glossary/B>

<sup>11</sup> <http://www.epa.gov/oem/content/learning/booms.htm>

## 2.4.5 Oil Sorbent Boom Technology



*“The Deepwater Horizon response has included the largest deployment of boom in the history of spill response” [27].*

Sorbent or “sorb” boom, as it is generally referred to in the oil industry, is designed to capture limited amounts of oil at the surface of water. This tool can be combined with a containment boom as a secondary line of defense, as commonly utilized in the Gulf to help protect coastal marshes. The cleanup effort during the Deepwater Horizon oil spill resulted in the deployment of more than 3.4 million meters of polypropylene filled sorbent boom [27]. In ideal cases, the spent oil-soaked sorb boom is sent to a cogeneration facility and burned for energy (waste-to-energy). Cogeneration, also known as combined heat and power, uses the heat produced during combustion of a fuel source, such as wood, to generate electricity. The only cogeneration in the state of the Louisiana occurs at small, on-site systems operating at sugar cane refining facilities [83].

The Deepwater Horizon spill was unique because the oil first traveled upward through a 1,500 meter water column and moved along a Gulf loop current while some of the oil eventually traveled 90 kilometers toward shore, confronting the sorb boom [84]. The 3.4 million meters of spent sorb boom was mostly sent to local Class 1 landfills for disposal [85]. Class 1 landfills are permitted to receive materials which are classified as non hazardous, but have the potential to produce a leachate. Therefore, all spill related waste was tested and designated as non-hazardous before being disposed of in landfills.

## 2.5 Oil Spill Waste Management

Louisiana has established regulations for promoting waste diversion practices due to limited space available in solid-waste landfills. The state had a previous influx of more than 22-million tons of disaster debris waste in the aftermath of Hurricane Katrina, the largest natural disaster in US history, and recognized the need to reduce materials entering landfills [86]. Louisiana State Senate Bill 583 was created as a comprehensive debris management plan with the goal to “reuse and recycle material, including the removal of aluminum from debris, in an environmentally beneficial manner and to divert debris from disposal in landfills to the maximum extent practical and efficient which is protective of human health and the environment” [87]. Senate Bill 583 prioritizes waste management practices for debris in this order: “recycling and composting; weight reduction, volume reduction; incineration or cogeneration and land disposal” to the extent they are “appropriate, practical, efficient, timely, and have available funding” [86].





**Figure 2.5:** Cleanup workers disposing of oily sand. Image courtesy of Patrick Kelley, U.S. Department of Defense.

Detailed waste management plans were created to outline disposal methods to ensure that the disaster waste was properly handled (Figure 2.5). The EPA, the Coast Guard, the Unified Area Command, and the Gulf States directly affected by the spill approved the waste management and disposal plans [87]. The waste generated from oil spill can be separated into categories depending on the composition of the material. These categories include solid waste, recovered oil, oily water and liquid waste, and animal carcasses. Solid waste is oil-contaminated material such as sorbents, debris and personal protective

equipment, as well as non-contaminated solids, such as those materials required by the support cleanup operations. In total, the Deepwater Horizon oil spill generated more than 70,000 tons of oily solids and 9,000 tons of solid waste, which was disposed of in Louisiana, Mississippi, Alabama and Florida municipal solid waste landfills [85].

## 2.6 Occupational Health and Safety

*“Those reporting exposure to oil and dispersants had significantly higher prevalences of upper respiratory symptoms and cough than those not exposed. Symptoms related to heat exposure were the most frequent in all groups” [88]*



**Figure 2.6:** Marsh cleanup workers - Barataria Bay. Image courtesy of PJ Hahn, Plaquemines Parish.

Studies of tanker oil spill responses have reported adverse health effects in response workers [89-92]. These studies may underestimate the health effects on the Deepwater Horizon response personnel because the spill’s magnitude and duration were unprecedented. Fresh oil generally is more toxic than weathered crude oil because the concentration of volatile organic compounds (VOCs) decreases with weathering. Still, weathered oil contains harmful compounds that can cause irritant reactions, and there is a potential risk for oil to be

aerosolized, or dispersed in air, in respirable airborne droplets or volatilized by cleanup activities such as raking oiled vegetation and pressure washing (Figure 2.6). A pressure washer is a high-pressure mechanical sprayer used during the

Deepwater Horizon spill to remove oil from containment boom for the purpose of redeployment [93]. Even though detection of hydrocarbon odors is common in areas contaminated by oil, odor is not a reliable indicator of health hazard. Some individuals are bothered by odors and can develop symptoms requiring medical evaluation [94]. Overall, there is an incomplete understanding of the cumulative human health hazard associated with the particular characteristics of this spill, including a very large volume of continuously-flowing oil, extensive dispersant use, and in-situ burning.

According to the Louisiana Department of Health and Hospitals, from April 25 to September 18, 2010, there were 411 reports of health complaints believed to be related to exposure to pollutants from the oil spill [95]. Of the total health-related complaints, 325 came from response personnel and 86 from the general population [93]. The most frequently reported symptoms were headaches, dizziness, nausea, vomiting, weakness/fatigue and upper-respiratory irritation. Due to a lack of chemical-specific air monitoring, especially for cleanup workers in vessels, direct correlations between chemical exposure and health complaints cannot be determined<sup>12</sup>. For example, the EPA's air monitoring at several fixed sites used a technology known as photoionization detection that can only measure total VOC, not specific compounds, such as benzene, toluene, ethylbenzene, and xylene [24]. According to the EPA monitoring plan, no samples were obtained from vessels in which cleanup workers were present. Sampling was primarily conducted onshore or by aircraft [88].

## **2.7 Lessons Learned from the Deepwater Horizon Response**

The offshore oil industry as a whole includes not only the primary corporations responsible for the majority of oil exploration, extraction and refining, such as Shell, Exxon-Mobile and British Petroleum, but also a multitude of contractors who supply, service and support the industry. British Petroleum and a joint-industry group, representing several of these major oil companies, published separate "lessons learned" reports in response to the Deepwater Horizon incident [4, 27]. The joint-industry group was led by Shell Oil, and was referred to as the Joint Industry Oil Spill Preparedness & Response Task Force [4].

The joint-industry report concluded that, "Subsea dispersant application significantly reduced the size of the surface slick and consequently reduced the shoreline impact" [4]. The British Petroleum document concluded that the decision to use dispersants, "may have been the most effective and fastest-mobilized tool for minimizing shoreline impact" [27]. The reports do not, however, present any empirical evidence as to the efficacy of the use of subsea or surface applications of

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<sup>12</sup> Centers for Disease Control and Prevention, Deepwater Horizon informational website <http://emergency.cdc.gov/gulfoilspill2010/>

chemical dispersants. British Petroleum is currently funding chemical dispersant research which includes studies to better understand the fate, toxicity and efficacy of chemically dispersed crude oil.<sup>13</sup>

It is also notable that the British Petroleum report refers to subsea use of dispersant as a containment innovation rather than a response technology. Containment, as defined by the joint industry report, is “the effort to disperse, cap, close and ultimately stop the release of hydrocarbons at the source” [4]. In this context, subsea dispersant use is described as a tool for preventing oil from coming to the water surface where response personnel would be employed to remove the oil from the environment. Their conclusion can be summarized in the following quote in the report, “The primary strategy should be to address the spill as close to the source (and as far offshore) as possible first controlling the subsea spill, then, applying appropriate quantities of dispersants” [4]. “Lessons learned” reports are important as they serve as a reference for decisions related to future policy-making and current regulatory oversight. Conclusions from these reports also provide an industry perspective for improving or changing oil spill response. The reports cite changes to the regulation and techniques related to controlled in-situ burning over the past ten years as an example of the type of deregulation that should be done for regarding the application of chemical dispersants.

To quantify the long-term impacts of oil spills it is important first to identify all variables that directly contribute to the development of a comprehensive assessment, so that all relevant environmental conditions are considered. An assessment requires access and participation of scientists from academia and government agencies. Understanding the impact on the environment of the Gulf due to the oil spill is important for informing future policy and decision-making related to risk management, fine assessment, and appropriate oil spill response.

Three key components of the Gulf oil spill’s initial environmental impact that should be included in the natural resource damage assessment [4]:

- **Natural Gas:** Natural gas currently is not considered an environmentally-harmful component of the total petroleum discharge. Including methane and other hydrocarbon gases, the total petroleum discharge amounted to more than 6.9 million barrel-of-oil equivalents, 2 million of which were natural gas [3]. The fate of the released methane, which biodegrades relatively slowly in water compared to the other components of natural gas and many liquid hydrocarbons, still is unknown [3, 84].
- **Waste Produced:** Oily and non-oily waste materials from cleanup efforts are disposed in local landfills and/or incinerated. These waste materials include one million meters of absorbent boom, oil,

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<sup>13</sup> Gulf of México Research Initiative, <http://gulfresearchinitiative.org/research>

sand, and sediment from shorelines, marine animal carcasses, personnel materials such as protective clothing<sup>14</sup> and gloves, vegetation, and other debris [96]. In addition, after the oil spill recovery operations ended, the response vessels and equipment had to be decontaminated creating additional oily wastewater [96, 97].

- **Response Efforts:** Decisions not to prioritize a “contain and recover” protocol most likely will have measurable impacts on the Gulf ecosystem [47, 98]. The toxicity of chemically dispersed oil and chemical dispersants released into the ocean, as well as air pollution resulting from in-situ burning of oil, are additional quantifiable environmental impacts [54, 68, 73].

Oil recovery completely removes oil from the marine environment therefore decreasing toxicity to the local ecosystem [28]. In contrast, chemical dispersion does not remove oil from the water but changes its distribution from the surface to the water column [31, 34, 74]. The addition of chemical dispersant and the degree to which it contributes to the absolute toxicity of an oil spill to marine life also is unknown [12, 31, 54]. In-situ burning removes most of the oil from the water surface, but leaves behind a dense residue which may or may not be possible to collect [69]. It also presents an occupational hazard to response personnel due to harmful gases and particulate matter released from the burn [68, 74]. These technologies often are employed simultaneously, resulting in a probable decrease in total recovery of oil. For example, surface oil is pooled prior to ignition, meaning it could be skimmed rather than burned. Similarly, applying chemical dispersants at the surface makes skimming, which requires a slick of oil, impossible [79]. Applying chemical dispersants at depth, most likely, reduces the amount of oil that reaches the surface and, again, reduces the potential for recovery [58].



**Figure 2.7:** An environmentally preferable oil spill response plan is one that has a lesser or reduced effect on human health and the environment.

<sup>14</sup> Tyvek or “plastic” suits, are worn by cleanup workers as a protective barrier for skin and clothing when working with hazardous substances, such as hydrocarbons and chemical dispersants.

An alternative oil spill response prioritizes containment and recovery as an environmentally preferable effort throughout successive stages of a spill or discharge (Figure 2.7) [84]. The first stage consists of capturing the oil using containment booms and recovering it with skimmers and absorbent booms—and considering the use of natural-fiber sorbents whenever feasible to reduce overall solid waste. The second stage, which begins when oil recovery efforts are working at capacity and moving oil presents an imminent threat to shoreline ecosystems, utilizes chemical dispersants and in-situ burning where necessary, while continuing containment and recovery. By minimizing chemical dispersant use and in-situ burning, and by prioritizing contain and recover techniques, adverse environmental effects of the cleanup response are minimized [28].

Current oceanic oil discharge environmental impact assessment is limited because of a lack of available data on both immediate and long-term effects [99]. The environmental issues previously discussed are quantifiable and, therefore, should be included in the environmental impact assessment of the Deepwater Horizon oil spill. Environmental impact assessments should include the discharge of natural gas, the disposal of waste materials related to the spill and its cleanup, and the environmental impacts of cleanup technologies, such as chemical dispersant application and in-situ burning. Oceanographic science response plans should be available to guide responder strategy in terms of gathering appropriate water column and sediment data. If independent scientists had not discovered the oil plumes and weathered, sedimented oil on the seafloor during the Deepwater Horizon spill, those features would not have been properly documented [55]. By having these data available, these phenomena can be quantified and included in comprehensive environmental impact assessments.

The release of natural gas contributes to the adverse environmental impacts of the spill and should be included in the total petroleum discharge calculation [3]. The disposal of a significant volume of waste material resulting from the oil cleanup impacts local landfills by introducing oily waste and adding to the overall material burden on solid waste disposal facilities. The material burden on waste facilities can be significantly decreased by selecting sustainable cleanup technologies, such as natural fiber absorbents, which currently are available. The benefits of chemical dispersant application should continue to be researched and weighed against the potential adverse effects. In addition, the net result of subsea dispersant use in the Deepwater Horizon spill still is unknown and should also be reassessed through controlled research. In-situ burning was used extensively, moving oil pollution from the water to the atmosphere, which presents a health risk primarily to response personnel and adds to global stratospheric pollution.

A report by Petroleum Finance Company Energy, cited by Lamar McKay of British Petroleum during the 2011 Offshore Technology Conference in Houston Texas, projects that wells in ultra-deep water, 1,500 meters depth or greater, will supply half of Gulf of México oil production by 2020 [100, 101]. If so, investment in oil containment and recovery technologies will be key to managing the unique risks

posed by ultra-deep water drilling to ensure safe oil production in the future. The facts presented in this chapter suggest a need for containment and recovery of oil to be prioritized whenever possible, and for a reconsideration of a more inclusive environmental impact assessment. The lessons learned from the Deepwater Horizon event also demonstrate a need to increase extensively the efficacy of existing oil spill response technology. If oil recovery technology is not adequate to manage oil near the incident site, as evident by the extensive use of dispersants and burning during the Deepwater Horizon spill, then additional investment should be made in technology which supports oil skimming. The net environmental benefits of spill response tools which do not contain or recover oil from the environment, such as chemical dispersants and in-situ burning, are not clear and therefore warrant further investigation.

## Appendix A. Acronyms & Key Terms

### A.1 Agencies and Actors

BOEMRE	Bureau of Ocean Energy Management, Regulation and Enforcement
BSEE	Bureau of Safety and Environmental Enforcement
EPA	U.S. Environmental Protection Agency
MMA	Minerals Management Agency
NOAA	National Oceanic and Atmospheric Administration, U.S. Department of Commerce
USCG	United States Coast Guard

### A.2 Deepwater Horizon and Emergency Response Terms

FOSC	Federal On-Scene Coordinator
ICS	Incident Command System
NCP	National Contingency Plan
NRT	National Response Team
OSCA	Oil Spill Cleanup Agents
RP	Responsible Party, British Petroleum
UC	Unified Command

### A.3 Laws and Regulations

CWA	Clean Water Act
HAZWOPER	Hazardous Waste Operations and Emergency Response regulations, 29 CFR 1910.120
OPA	Oil Pollution Act

### A.4 Key Terms

Biodegradation - occurs when micro-organisms, such as bacteria and fungi, feed on oil as a source of carbon.

Emulsification - a process that forms *emulsions* consisting of a mixture of small droplets of oil and water. Emulsions are formed when wave action and strong currents causes water to become trapped inside viscous oil.

Evaporation - occurs when the lighter substances within the oil mixture become vapors and leave the surface of the water. This process leaves behind the heavier components of the oil, which may undergo further *weathering* or may sink to the

ocean floor. Wind, waves, and currents increase both evaporation and natural dispersion.

Oxidation - occurs when oil contacts the water and oxygen combines with the oil to produce water-soluble compounds.

Weathering - a series of chemical and physical changes that cause spilled oil to break down and become heavier than water. Winds, waves, and currents may result in natural *dispersion*, breaking a slick into droplets which are then distributed throughout the water.



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# Chapter 3

## Deepwater Horizon Oil Spill Remediation: Remediation and Restoration of Northern Gulf of México Coastal Ecosystems

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### 3.1 Introduction

Upon surfacing, oil from the blown Macondo well was transported across the northern Gulf of México, where it grounded on shorelines from Florida to Louisiana [1]. Within nine days of the explosion on the DWH drilling rig, oil entered Louisiana wetlands at the mouth of the Mississippi River [2]. Within a month, oil had coated shoreline beaches and wetlands throughout the Mississippi River Delta, the largest coastal wetland complex in the continental United States [2]. By the time the disabled well had been capped, oil had grounded on shorelines throughout the northern Gulf of México, including sensitive wildlife refuges like the Chandeleur Islands in Breton Sound, and white sand beaches frequented by tourists in Florida, Alabama and Mississippi [2]. The heaviest accumulations occurred in Louisiana as a consequence of currents and prevailing winds directing much of the oil to the west of the mouth of the Mississippi River [3]. As in other states, oil grounded on to barriers island beaches, but much of the shore-bound oil penetrated into Mississippi River Delta wetland ecosystems. Oil entered marsh and mangrove habitats from the Bird's Foot Delta to Terrebonne Bay, including areas located miles inland from the ocean [3]. As of 20 January 2011, surveys of more than 4,000 linear miles of the northern Gulf of México coast, conducted for the pre-assessment phase of the Natural Resource Damage Assessment (NRDA), documented 1,053 miles of oiled shoreline<sup>1</sup>.

Coastal ecosystems of the northern Gulf of México encompass many of the most productive and biologically important habitats in North America [4]. In addition to supporting sensitive resident species like the Brown Pelican, these areas shelter the majority of overwintering waterfowl that travel the Mississippi Flyway [5]. Northern Gulf of México coastal ecosystems also provide regulatory services such as storm protection, water filtration and nutrient capture; provisioning services like fin-fish and shellfish fisheries; and cultural services including heritage tourism, recreation, and aesthetic value [6]. Coastal habitats (e.g. oyster reefs and marshes) in Louisiana alone support 30% of US fisheries production, and it has been estimated that Mississippi River Delta ecosystems generate at least \$12-47 billion in

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<sup>1</sup> <http://www.gulfspillrestoration.noaa.gov/oil-spill/gulf-spill-data>

annual benefits [6]. As an economic asset, the Delta has a minimum value of \$330 billion to \$1.3 trillion, with 90% of its value attributable to services derived from wetlands [6]. Oil exposure has placed the ecological and economic well being of the northern Gulf region at risk by potentially affecting many, if not all, of the valued services provided by these coastal ecosystems.

The federal government, state governments, and the responsible party (British Petroleum Plc.) mounted a vast and complex response effort soon after oil from the Macondo well was detected in offshore waters [7]. Responders were required to make difficult choices among possible interventions, including what steps to take to prevent oiling of shorelines and removal of oil from sensitive coastal ecosystems. Responders had to decide, for example, whether to contain and recover oil via skimming technologies versus chemically dispersing and burning hydrocarbons from the surface of the water. Experiences during prior oil spills have led to a general understanding that response actions can cause more harm than good [8]. Pressurized hot-water washing of oiled rocky intertidal shorelines during the Exxon Valdez oil spill (EVOS), for example, likely induced greater macroalgal and invertebrate mortality than did exposure to oil [9]. Even though consideration is now given to the possibility of unintended outcomes, imperfect knowledge of trade-offs between potential benefits and risks from interventions have nonetheless complicated DWH response efforts [10, 11].

As in the EVOS, protection and remediation of oiled northern Gulf of México shoreline ecosystems has involved weighing potential benefits against the risk that interventions intended to reduce damages from oil exposure will instead lead to further injury. Oil removal from coastal wetlands, for example, can reduce acute and chronic exposure of both resident and migratory species, but many traditional removal approaches can cause immediate and enduring damage to fragile soils and sensitive wetland biota [12-14]. Simply setting foot in salt marshes can result in soil compaction and loss of foundational plants, which can accelerate erosion and lead to permanent loss of marsh habitat [15]. Surface application of dispersants, as was done across northern Gulf of México waters, can reduce shoreline oil accumulations but it adds petroleum-based products into areas that serve as nursery habitat [16]. Some interventions, such as diversions from the Mississippi River, can involve protection of one ecosystem at the expense of another [17]. Freshwater diversions intended to provide counterbalancing flows to prevent oil from entering delta wetlands may have collaterally damaged nearby oyster grounds sensitive to low salinity conditions [17]. Consequently, oyster grounds were exposed to the combined influence of oil and freshwater exposure during peak spawning periods, which may have resulted in greater injury to future harvests (ie. by elevating larval mortality and depressing adult reproduction) than complications from oil exposure alone [17]. Thus, some trade-offs can also endanger the socioeconomic well-being of communities including cities like New Orleans that depend on coastal ecosystems for income and security [18].



Despite the possibility of unintended outcomes, interventions had to be carried out to prevent acute and chronic oil exposure of sensitive biota to oil. As of early 2011, the consolidated fish and wildlife collection report maintained by the US Fish and Wildlife Service (USFWS), which provided daily updates on the number of injuries and deaths of vertebrate species of concern, listed 2,630 injuries and 6,833 mortalities resulting from oiling [19]. In comparison to similar counts following the EVOS disaster, it appears that the Gulf has sustained relatively low levels of damage from the Macondo well blow out [17]. Acute damages are far less than what many feared would result from the massive release of oil, but little is known about damages that emerge over long time spans [20]. For example, lags can emerge if reproduction is depressed by chronic, sublethal exposure or reduced resource availability due to ecosystem-wide disruption of food webs [21].

The persistence of oil in coastal environments more than a year after the DWH blowout indicates that Gulf of México biota remain susceptible to acute and chronic exposure [22]. At the beginning of 2011, the Shoreline Cleanup Assessment Technique (SCAT) program reported that 336 of 1,053 miles of oiled shoreline warranted treatment, and that at least 83 miles remained heavily to moderately oiled [21]. Surveys of Louisiana embayments conducted by independent researchers also found that oil has persisted under heavily matted vegetation in Barataria Bay marshes, especially in areas where larval and water surfaces are not exposed to weathering [23]. Impervious rinds also have formed on some surfaces exposed to weathering, which can slow aeration and inhibit microbial activity [24]. A survey of beaches on the barrier island chain fronting Barataria Bay found evidence of buried oil in cohesive layers  $\geq 20$  cm thick covered by 10-80 cm of clean sand above the water table and vertically diffuse 10-50 cm thick bands of oil below the water table [25]. Oiled sand reworked by wave action has also coalesced into subtidal tar mats in surf zone depressions that could extend for miles off of some areas of the coast, such as Perdido Key beach on the Florida panhandle [26].

It is now widely recognized that many of the most pressing questions about shoreline impacts and recovery remain unanswered. Created by President Obama as an Executive Order<sup>2</sup> on 5 October 2010, the Gulf Coast Ecosystem Restoration Task Force has been tasked with addressing this concern by promoting the development of more effective shoreline remediation strategies. Two key conditions have been identified for redressing shoreline damage from the DWH blow out. First, approaches must be science-based. Second, approaches must address oiling, erosion and subsidence. Oil from the blown Macondo well grounded on areas of the Gulf coast that are experiencing high rates of habitat loss as a consequence of erosion and subsidence [1]. Marshes in Barataria Bay and other heavily degraded deltaic wetlands, for example, are hotspots of habitat loss [27]. Back-of-the-envelope estimates suggest that oiling could increase annual habitat loss by 30% (LUMCON, unpublished data), with most of the additional loss concentrated in

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<sup>2</sup> Executive Order #13554 – Gulf Coast Ecosystem Restoration Task Force  
<http://www.whitehouse.gov/the-press-office/2010/10/05/executive-order-gulf-coast-ecosystem-restoration-task-force>

highly susceptible wetlands that provide valuable ecosystem services. Thus, response strategies that only address oiling likely will not result in permanent gains.

Here we assess the prospects for achieving and implementing a forward-minded response policy of post-spill habitat remediation and restoration. Focusing on Louisiana coastal marshes that received the heaviest accumulations of oil, we first review the formulation and execution of conventional response strategies for shoreline protection and remediation. We then examine how novel approaches were evaluated and implemented, including several controversial interventions undertaken to protect sensitive coastal ecosystems during the DWH spill. We also overview the down-selection process of shoreline clean up approaches with reference to studies aiming to improve the process and outcomes of shoreline remediation. Finally, we identify steps that could be taken to promote ecosystem recovery by linking shoreline remediation with habitat restoration, placing emphasis on local sourcing and novel approaches that reduce operational trade-offs and maximize efficiencies.

### **3.2 Oil Spill Response Administration and Structure**

A National Contingency Plan (NCP) serves as the federal government blueprint for responding to oil spills in federal waters. In accordance with the plan for coastal zones, the United States Coast Guard (USCG) was charged with overseeing the DWH oil spill and appointed a National Incident Commander after it was declared a Spill of National Significance (SONS) by the Secretary of Homeland Security [28][28]. The Incident Command System (ICS) provides the framework necessary for coordinating the effort of the government agency response organizations [3]. The agency organizational structure in the spill response, from top to bottom, includes:

- National Response Team (NRT) – consisting of 15 federal departments and agencies including the USCG, the National Oceanographic and Atmospheric Administration (NOAA), the Department of Interior (DOI), and the US Environmental Protection Agency (EPA)
- Regional Response Teams (RRTs) and Rapid Assessment Teams (RATs) – consisting of clean up operation staff led by the USCG and EPA, which has authority over the use of dispersants
- Area Committee (AC) – consisting of local government and environmental agency representatives

As oil came ashore, RRTs commenced local cleanup operations, guided by pre-established Area Contingency Plans (ACPs). Cleanup proceeded in stages following the SCAT process, using surveys and assessments to create stage-specific Shoreline Treatment Recommendations (STRs) [29]. The RRTs deployed operations task forces to conduct cleanup activities using remediation techniques described by STRs

until the segment was judged to require ‘no further treatment’ (NFT) [30]. Evaluations were made according to group consensus amongst members of a SCAT team, requiring agreement between representatives from federal, state, and sometimes local government or other shareholders, as well as the team lead and representatives of BP [30]. The transition from cleanup to long-term recovery follows the Shoreline Cleanup Completion Plan (SCCP) as a framework for providing the final definition of NFT for each shoreline type [30]. The SCCP was written collaboratively between the USCG, NOAA, DOI, the Gulf States and BP, although the State of Louisiana refused to sign [31]. Having the authority to make response decisions, the USCG federal on-scene coordinator nonetheless enacted the SCCP on 2 November 2011 [32].

### 3.3 Limitations of Shoreline Protection and Conventional Onshore Treatment

The sheer magnitude of the DWH surface spill and limitations of offshore prevention and containment measures required implementation of measures to remediate oil contaminated shoreline [14]. Stage I and II shoreline cleanup responses were implemented to treat moderately to heavily oiled shoreline in danger of being repeatedly oiled while the wellhead was leaking [14]. SCAT teams created general STRs for Stage I and Stage II responses according to habitat categories including sand shoreline, coastal marshes and mangroves, and manmade shoreline [14]. After the Macondo well was capped, SCAT teams shifted to Stage III responses to treat oiled shoreline [33]. Stage III guidelines were based on SCAT Core Group concerns and Taskforce Working Group recommendations for different habitats [30]. Site-specific STRs were also created with the goal of reducing oiling levels to enable natural attenuation [33].



**Figure 3.1:** Smooth cordgrass, Bay Jimmy Louisiana, October 2011.

Response methods were selected according to the intensity and form of oiling as well as potential treatment impacts [30]. Strategies were guided by concepts underpinning Net Environmental Benefit Analysis, where responders clearly recognize what can be achieved before treatment actions become unsafe, impractical, give no significant benefit, or could start to cause further damage to a shoreline habitat/resource resource [33]. For sand shorelines, which represent perhaps the simplest logistical conditions for shoreline treatment, responses largely involved removal, tilling, and sifting of sand by crews supplemented with industrial scale equipment like ‘Sand Sharks’ [30, 33]. Sand was also cleaned in treatment

plants and returned to affected shorelines [33]. Coastal marsh habitat presents significantly more challenging conditions for treatment as a consequence of soil and biotic structural complexity [33]. Although oiling mostly occurred along peripheral edges, oil penetrated tens of meters in to marsh interiors at some locations, where foundational vegetation was coated to heights ranging from a few centimeters to over one meter due to tidal flux [14].

Thick layers of oil were found trapped in dense stands of vegetation, underneath organic debris (e.g., wrack), and on soil surfaces [33, 34]. Oil also grounded on to root surfaces, which can prevent oil from penetrating deeply into soils. Guidelines for STRs and NFT under the Stage III Shoreline Treatment Plan recognized that treatment of sensitive marsh environments could cause physical harm significantly more detrimental than consequences solely attributable to oiling [33, 34]. The primary response recommended for oiled marshes was natural attenuation, whereby oil would be physically removed by wave action and tides or natural degradation through microbial metabolism and photooxidation [33]. Initial plans nonetheless identified a limited set of possible treatment options (depending on site conditions), which included low-pressure or ambient-temperature flushing, contained sorbents, manual removal, vacuuming, vegetation cutting, and natural recovery [33].

Implementation of available treatment options for coastal marshes proved problematic. Low-pressure, ambient-water flushing, which was permitted from vessels operated from the marsh edge, was not effective against heavy accumulations of fresh and weathered oil [33]. Low-pressure flushing techniques were also recommended for use only when tides covered marshes because spray turbulence could suspend sediment or spread contamination into soils [33]. This technique also saw little use because of limited availability—in Louisiana, only crews from St. Bernard Parish had access to proper equipment [33]. Contained sorbents, typically made of polypropylene, were used on water surfaces to recover oil being released from adjacent shoreline [33]. Limited surface area and the adsorbent nature of the boom provided little capacity for use against light sheens. Improperly monitored boom also stranded in marshes, spreading contaminants, creating debris, and causing damage. Manual removal of oil was constrained by limited access and damages resulting from foot traffic—even light foot traffic can compact soils and cause significant long-term harm to resident biota in marshes.

Consequently, manual oil removal was restricted to areas of marsh with firm sand or shell substrate, where hand tools such as trowels and shovels were used to remove thick accumulations [33]. Because of potential risks to sensitive shoreline, response teams typically only completed partial treatment through manual removal. Similar concerns restricted implementation of portable vacuum treatments to partial removal of oil from marsh shoreline; vacuums could not be operated from an offshore vessel without potentially disturbing and removing soil and sediment [33]. Cutting and removing oiled vegetation and organic debris, often with string trimmers and blades, was considered to be too aggressive to serve as a primary

response approach. It was, however, permitted on a case-by-case basis for recovering oil trapped in thick stands of *Phragmites australis*. Initial treatment plans prohibited cutting *Spartina* cordgrass and mangrove vegetation [33].

Several treatment methods were identified as being of little potential value because of limited applicability against weathered oil or because oiled materials could not be recovered from the environment. These included deluge flooding, solidifiers, loose sorbent materials, and surface cleaning agents [33]. In-situ burning, where tidal flooding allows for plant regrowth by protecting roots from heat, would have been considered an appropriate remediation tool if the oil had been ignitable and floating freely in marshes [33]. Fertilizer additions to promote microbial metabolism and breakdown of oil were also ruled out because northern Gulf coast marshes are not nutrient limited environments [33]. Methods specifically not recommended for vegetated shoreline included mechanical oil removal, sediment reworking/tilling, and any kind of high-pressure or heated water flushing [33]. These methods were determined to be too destructive because of the likelihood that oil would penetrate further into porous sediment; that substrates would be compacted; or that plants or soil microorganisms would be damaged [33].



**Figure 3.2:** Oiled marsh shoreline; April 14<sup>th</sup>, 2010, Barataria Bay, Louisiana.

The DWH Shoreline Treatment Implementation Framework incorporated guidance and recommendations to minimize potential harm from treatment approaches, citing research literature, agency protocols, and previous oil spill experiences compiled by the SCAT Taskforce Working Groups. The Framework outlined appropriate Stage III Shoreline Treatment Recommendations and No Further Treatment goals, and was approved by Core Groups made up of stakeholder representatives. Nonetheless, SCAT teams developed STRs that strongly deviated from the Implementation Framework, and the UAC approved the use of aggressive strategies to remove oil from sensitive ecosystems.

The cleanup of marshes in Bay Jimmy (Barataria Bay, Plaquemines Parish, Louisiana), which may have received more oil than any other vegetated shoreline during the DWH event, offers exceptional examples of how cleanup crews implemented aggressive treatment strategies. Across Bay Jimmy, vegetation laid down by waves became trapped under the weight of oil, creating tarry debris mats (Figure 3.2). Heavily oiled wrack lines subsequently hardened into tarry asphalt. Thick emulsified oil, or mousse, pooled on soil surfaces and became trapped beneath matted vegetation and wrack, preventing degradation. Tidal flushing and compaction nonetheless released buried mousse from exposed marsh. Few options are available to remove heavy accumulations of weathered oil without disrupting

vegetated shorelines. The ineffectiveness of conventional treatment approaches, and the threat of additional resources suffering damages from oiling (i.e. contaminants could persist and potentially spread throughout the embayment), prompted consideration of aggressive tactics for remediation of Bay Jimmy shoreline. Following completion of a study to assess potential outcomes (described below), aggressive measures were implemented to recover oil trapped in marsh soils, vegetation and debris that would have otherwise remained heavily contaminated [33].

In Northern Barataria Bay, 11 km of shoreline were treated using aggressive raking and cutting [33, 34]. Cleanup crews entered marshes, provided that boards were laid down to serve as temporary walkways. Thick mousse was scooped out with shovels. Heavily oiled wrack lines and vegetation mats were raked and removed through mechanical equipment (Figure 3.3). Raking and cutting often continued until only stubble remained in place. Force was often required to break through hardened surfaces to reach oiled mats below [34]. The difficulty of penetrating tarry surfaces to remove oiled debris required mechanical tools like hedge trimmers and chain saws [34]. A provision was made to allow heavy equipment to be used on a case-by-case basis to scale up treatment and improve response efficiency [34]. Long hydraulic arms operated from barges or airboats to reach into the marsh with automatic raking, cutting and excavation attachments allowed mechanization for crews to scale up aggressive treatment techniques. Heavy machinery also enabled transfer debris directly from the marsh to offshore disposal containers, but the equipment had less precision than manual recovery and could damage marsh soils. Contaminated soil and sediment, including horizons dominated by organic content, were excavated until clean sediment was uncovered [34].



**Figure 3.3:** Aggressive remediation of oiled shoreline in Bay Jimmy (Barataria Bay, Louisiana). Cleanup crews manually raking oiled debris and cutting oiled vegetation (left); mechanical removal of oiled material (right). Photos courtesy of P.J. Hahn.

Seasonal conditions prompted shoreline treatment to be expedited [34]. Access generally improves toward winter when tides decrease and substrates harden.

Removal of oil during winter also enables plants to recover the following spring before the beginning of hurricane season. However, delays exacerbated access and treatment conditions. Shoreline treatment in Bay Jimmy stretched into the summer of 2011, when wet conditions in the marshes increased susceptibility to damage [34]. Yet the approaching hurricane season, which raised concerns that unrecovered oil could become resuspended and redistributed, spurred ambitious STRs for Northern Barataria Bay with crews implementing aggressive removal approaches. By the end of September 2011 shoreline remediation crews had manually or mechanically removed over one million pounds of material from Bay Jimmy marshes [34].

Remediation of vegetated shoreline was further complicated by the nature of on-site reviews of treatment outcomes. Meeting NFT Guidelines under the STR for each site requires unanimous agreement between a federal representative (usually NOAA, a NOAA contractor, or USCG), a state representative, and a BP representative on each SCAT team (a landowner may also be involved) [34]. Representatives sometimes disagree, though, as to what constitutes cleaned shoreline. Variable experience and training can contribute to differing perspectives, including the amount of emphasis placed on risks posed by toxicity and exposure.

Attempts to achieve consensus potentially pushed teams (i.e. particularly those that included determined members) to err on the side of over-treatment, extending well beyond STRs. Yielding to the assumption that aggressive removal of oil and exposed debris provides greater certainty of net environmental benefits arguably reflects the difficulty of quantifying potential impacts of remaining debris or impacts arising from treatment.

### **3.4 Advancement through Failure & Innovation**

The 1989 EVOS in Prince William Sound (Alaska) exposed troubling limitations in response technologies and approaches, including the design and implementation of chemical dispersants and shoreline cleaning agents [21]. The EVOS resulted in passage of the Oil Pollution Act (OPA) in 1990, which created an interagency committee responsible for coordinating oil spill response research and technology development. Adopting the principles established by OPA, some states (including California and Alaska), now explicitly require that oil spill response make use of the best available or “achievable” technologies [35]. Under the OPA, spill response is intended to keep pace with advances in oil and gas exploration through a system of exercise drills, specialized training, and contingency planning [30]. Yet response improvements have largely been motivated during oil spill events rather than from preparation between spills [36]. The logic of this is simple interim preparation and planning based on past spill events and potential contingencies will not necessarily reflect novel conditions emerging from unfolding events. Little innovation will

come from practice exercises and spill response training limited to a predetermined range of spill scenarios. The frequency and scope of exercises may also reduce the likelihood that innovations to improve deficiencies will emerge from planning and exercises [37]. In any given area of concern, exercises are held once every three years, and may only involve participation of one “responsible party”, which can prevent interactions among regulatory and industry partners (i.e. a port may have anywhere from 25 to 250 regulated entities) and limit knowledge of Area Contingency Plans (ACPs) [37]. Furthermore, few clear mechanisms exist following exercises for sharing lessons, best practices, or new knowledge (i.e. of corrective actions) gained by agencies and outside partners.

Given the structure of OPA, it is not surprising that the best available response technologies and approaches did not adequately address the range of conditions that emerged during the DWH event. The experimental use of dispersants is among the most widely recognized outcomes of the limited range of innovations that were achieved prior to the spill. The administrator of the US EPA described the novel use of dispersants as “somewhat trial and error”, with concerns ranging from the potential impact of the volume of dispersants applied, effectiveness of dispersants at low temperatures, oil weathering as it rose to the surface, and environmental effects of dispersant in deep ocean environments. Indeed, the Region 6 RRT Regional Integrated Contingency Plan lists one of the disadvantages of subsurface dispersant use as “lots of unknowns” [38].

### **3.5 Evaluation of Alternative Response Technologies**

Recognizing the need for innovation, the Unified Command implemented the NOAA-led Alternative Response Technology Evaluation System (ARTES) as response efforts proceeded during the DWH event. ARTES was developed to help identify viable spill-specific response tools through the evaluation of tools based on technical merit. Traditionally, ARTES only considers chemical and biological countermeasures, but the program was expanded during the DWH event to include mechanical countermeasures. The ARTES program consequently considered a range of technologies including oil sensors, booms, skimmers, decontamination and waste minimization technology, shoreline cleaning machines and source containment innovations [39].

The ARTES was modified during the DWH event to include four primary stages of review. There were four mechanisms for vendors wishing to introduce alternative technologies for use during the spill— Unified Command center walk-ins, website<sup>3</sup> submission, community meeting forums, and VIP<sup>4</sup> submissions. Technologies that

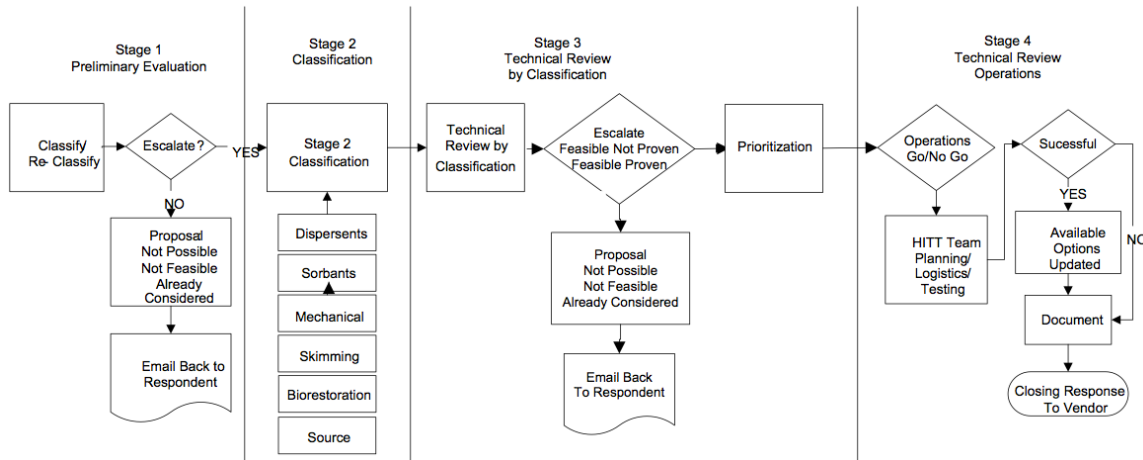
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<sup>3</sup> [www.horizedocs.com](http://www.horizedocs.com)

<sup>4</sup> Inputs received at Unified Area Command and Incident Commanders



passed Stage 3 review were considered for field testing and potential adoption (Figure 3.4). The VIP submissions were prompted by requests from high-ranking government officials or high profile individuals, or because the candidate technology garnered mass media attention during the course of the spill [7]. Of the ~123,000 submissions, approximately 100 reached Stage 4 field testing and only 25 technologies were adopted [39].



**Figure 3.4:** Alternative Response Technology Triage Process [7]

Some of the VIP technology submissions that were evaluated through ARTES included the following:

### 3.5.1 Human Hair Sorbent Boom

Alternative sorbent technologies, including human hair, were considered for oil adsorption as a consequence of public pressure arising from extensive media exposure of a grassroots effort orchestrated by the non-profit organization, Matter of Trust<sup>5</sup> to introduce the use of natural fiber as a filler material for sorbent boom. Media attention, which included interviews with the director of Matter of Trust by National Public Radio and the British Broadcasting Corporation, resulted in the donation of more than a dozen >10,000 square foot warehouses for storage and fabrication of hair booms across the Gulf coast [40]. Hundreds of pounds of hair were received daily during the height of media coverage, with volunteers working to fabricate sorbent boom. Field tests of the boom, carried out by BP near the Incident Command Center in Mobile, Alabama, revealed that it did not float and therefore did not meet established criteria for sorbent boom [40].

<sup>5</sup> [www.matteroftrust.org](http://www.matteroftrust.org)

### 3.5.2 A Whale Skimmer

A 1,115 foot Taiwanese freighter, built originally as an OBO (oil/bulk ore) carrier, was modified during the DWH event for oil skimming [41]. According to maritime reports, the ship was in Rio de Janeiro awaiting orders when it then traveled 4,240 nautical miles to a shipyard in Setabul, Portugal for skimming modification. A series of twelve 16-foot slots were cut in the forward hull of the ship, allowing oil-water mixtures to pass into existing internal tanks where oil would separate from water by gravity (Figure 3.5). The water would then be returned to the ocean and oil would be held on the vessel for transport to a shore side facility.



**Figure 3.5:** Oil skimming vessel, "A Whale". Image courtesy of US Coast Guard Petty Officer 1<sup>st</sup> Class, Jason Masson.

On 3 July 2010, BP, the US EPA and USCG conducted a test of the vessel's oil skimming ability. The USCG subsequently reported that *A Whale* recovered negligible amounts of oil. Limited to speeds of 2-3 knots, *A Whale* did not efficiently capture oil-water mixtures through its passive intake system [42]. Smaller Vessels of Opportunity were comparably more efficient and considered to be logistically more nimble than the modified freighter.

### 3.5.3 Costner Centrifuge

Blue Planet Water Solutions (BPWS) is a company founded by actor-director-producer Kevin Costner, which developed an advanced oil-water separation technologies for oil spill cleanup<sup>6</sup>. The company's foundation oil separation technology, which was transferred from the Department of Energy to Costner Industries Nevada Corporation (CINC) in 1993, is capable of highly efficient mechanical separation across a range of throughput conditions on board spill response vessels [43]. The BPWS liquid-liquid centrifugal separator unit utilizes the force generated from rotating an object around a central axis. Spinning two fluids of different densities within a rotating container results in the heavier fluid being forced to the exterior walls of the rotor and the lighter fluid being forced to the center. Separation of oil and water can yield water of up to 99.99% purity, depending on the nature of the oil and the quality of the receiving water body [43]. The BPWS Integrated Systems, which integrate centrifuge and membrane oil-water

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<sup>6</sup> <http://www.bpws.com>

separation with water purification, have been designed to handle small and large volumes of oil and water at speeds of 2-200 gallons per minute. Additionally, the separator units can handle changes in liquid ratios from 10-1 oil-water to 10-1 water-oil without any loss of efficiency. Performance also can be adapted (in real time) to fluctuations in flow rate, water quality and temperature.

With the assistance of Plaquemines Parish President Billy Nungesser, BPWS approached BP about carrying out field tests to evaluate potential applications of the company's liquid-liquid centrifugal separation technology [44]. The BPWS Integrated System was tested by BP in April 2010, after which engineers from BP and BPWS worked in concert to optimize the BPWS Integrated System to process recovered oil of various ages and in varying states of emulsification. After roughly a month of ongoing testing and optimization, BP leased 32 of the BPWS Integrated Systems, eight of which were installed on *Edison Chouest* platform supply vessels (Figure 3.6). A Deep Water barge from Hornbeck Offshore also was equipped with the BPWS Integrated Systems for further processing capacity (Figure 3.6).



**Figure 3.6:** Two BPWS Integrated Systems installed on the D&L Salvage barge *The Splash* (left) and four BPWS Integrated Systems installed on the *Edison Chouest* platform supply vessel *ELLA G* (right). Images courtesy of Professor Eric Hoek, UCLA.

Use of oil-water separation technologies can help to reduce the need for other conventional technologies and approaches - including chemical dispersants, burning boomed oil, and use of oil adsorbent media. Development and adoption of innovative treatment technology can increase the efficiency of oil spill recovery operations (e.g. improving the quantity and quality of recovered oil), while also addressing environmental concerns such as reducing hazardous waste disposal and discharging skimmed water that meets or exceeds clean water standards [45].

Ensuring that future spill responses make use of the 'best available technology' requires that incentives to innovate and technology review programs be maintained on a permanent basis. A collaborative effort between federal and state agencies is now underway to revise the structure of ARTES so that it is available between oil spill events, with the goal of continuously improving spill response technologies.

Nonetheless, it remains unclear whether sufficient incentives (e.g. grant programs, tax subsidies, industry safety regulations) will be emerge so that innovation and advancement of spill technologies does not wane.

### **3.6 Shoreline Interventions**

Actions taken to keep oil from grounding on northern Gulf Coast shorelines extended well beyond technology review and approval through the ARTES program. The demand for novel approaches and solutions to reduce risks of shoreline contamination increased with the growing magnitude of the DWH spill. Media and institutional pressure, sometimes from state and regional authorities, to protect shorelines resulted in major interventions being proposed and executed. The construction of temporary sand berms, restriction of tidal inlets, and diversion of Mississippi River flows were three highly controversial (i.e. of high risk and uncertain outcome) interventions executed to reduce the likelihood of oil entering sensitive coastal ecosystem.

#### **3.6.1 Barrier Sand Berms**

One month after the DWH rig exploded, the Louisiana Office of Coastal Protection and Restoration (OCPR) applied for a permit to build sand berm barriers to protect shorelines to the east and west of the Mississippi River outlet [46]. The purpose of the project was to move 20 million cubic yards of dredge sediment seaward to the existing barrier island system in an effort to mitigate inflow of oily seawater into the Mississippi Delta region [47]. The OCPR application argued that the berm structures could function as geomorphic obstructions capable of protecting sensitive coastal ecosystems far more difficult to remediate than sandy substrate [47]. The Louisiana Barrier Berm Oil Spill Response Project was approved by the Army Corps of Engineers on 2010 May 27, with the USCG instructing BP to provide \$360 million for construction of 74 km of sand berms on the Chandeleur Islands and from Scofield Island to Timbalier Island as part of the on-going oil spill response [47]. By 22 November 2010, however, only 20 km of berm had been completed according to the permitted plan.

Critics of the project—which was designed by a dredging company prior to the DWH event to help reduce saltwater intrusion into the delta—expressed concerns reflecting value, logistics and functional outcomes. At least one million cubic meters of material would be necessary to build 74 km of berms at the proposed 2 m height [47]. Suitable materials are limited in the areas where the berms were to be constructed [48], and much of the material in the areas of concern had already been identified for future barrier island restoration projects. Dredging of material in the

targeted areas also could have resulted in displacement or mortality of benthic biota. By reducing seafloor elevations, for example, dredging can potentially reduce areas used by benthic biota as refugia during seasonal periods of anoxia [49, 50]. Critics also argued against dedicating large amounts of response resources to temporary structures that might not function as expected [51, 52]. Sand berms are immediately susceptible to erosion from wave action, especially during hurricane season. Assimilation of the berms into the littoral budget of the protected islands was presented as a potentially positive outcome of the project, but critics viewed this as a suboptimal and costly use of limited resources [53]. Noting many of these concerns, the National Commission on the DWH Oil Spill and Offshore Drilling concluded that the berm project was arguably “the most expensive and perhaps most controversial response measure deployed to fight the Deepwater Horizon spill” [53].

### **3.6.2 Inlet Restrictions**

During the DWH event, inlets located between barrier islands or at the mouth of estuaries functioned as potential gateways for oil to cross into inland waterways and ground on to interior shorelines. Recognizing the potential for oil to pass through inlets, state and parish authorities in Louisiana proposed closing the mouth of Barataria Bay with rock and barges. Coastal scientists expressed tremendous concern in response to the proposal, indicating that the project could have lasting detrimental consequences. By reducing tidal-driven sediment and water exchange, restriction of inlets can profoundly alter the physiochemistry and biota of inland waters and ecosystems [1]. Changes in salinity, oxygen levels and turbidity of inland waters [53-55] can result in mass mortality of inland biota (e.g. fish kills). By increasing tidal flow velocity, inlet restriction also can promote scouring and loss of adjacent shoreline [56]. Although the permit request for the planned inlet closures for Barataria Bay was denied by the USACE, a similarly minded plan was executed on Dauphin Island at the mouth of Mobile Bay in Alabama. Referred to as the “Katrina Cut” project, geotextile tubes and riprap fill were used to close a gap in the island created by storm surge from Hurricane Katrina in 2005. The Katrina Gap closure was initially intended to be temporary, but it is now likely that the inlet will remain closed [53].

### **3.6.3 Freshwater Diversions**

Soon after the oil spill commenced, the State of Louisiana opened two diversions (with maximum discharge rates of  $150\text{--}200\text{ m}^3\text{ s}^{-1}$ ) to allow freshwater from the Mississippi River to flow into Barataria Bay and Breton Sound. Diversions had previously been carried out to regulate salinity conditions and to deliver sediment

for coastal restoration, whereas the intended outcome of the diversions during the DWH event was a countervailing force capable of preventing the flow of oil into inland waters and coastal ecosystems. No evidence is available that suggests the freshwater diversions prevented or reduced the flow of oil into inland waters and ecosystems [53]. It is now clear, however, that the diversions resulted in water quality conditions unfavorable to some of the coastal ecosystems, such as oyster reefs, slated for protection [18]. The productivity of oyster grounds exposed to elevated freshwater conditions is expected to be depressed for a subsequent period of at least three years [53].

Although the decisions to construct sand berms, fill inlets and divert freshwater may indicate otherwise, innovation in spill response will likely be a defining element of the DWH legacy [57]. The costly and controversial interventions undertaken during the DWH event underscore the importance of basing spill response strategies on sound scientific knowledge so that outcomes do not undermine long-term coastal management plans. Perhaps even more so than the EVOS, the DWH spill has promoted greater awareness and appreciation that logistical and technological challenges must be overcome while being mindful of ecological conditions to effectively recover and sustain valued ecosystem services provided by oiled shoreline habitats.

A growing body of research begun soon after the Macondo well blow out will undoubtedly help advance oil spill response in the Gulf of México and elsewhere. RAPID grant funding from the National Science Foundation (NSF) has supported studies of marine and coastal Gulf of México ecosystems. Although the policy of NSF RAPID funding is to provide time-sensitive support for basic science, many of these studies are intended to assess outcomes of oil being released into the Gulf of México. Studies intended to evaluate the influence of oil on the structure of salt marsh and estuarine food webs, for example, provide a basis for assessing whether species interactions and trophic cascades can extend the footprint of exposure beyond immediate contact [58]. Other studies intended to evaluate biogeochemical outcomes of carbon subsidies from oil degradation will help determine the potential for manipulating resources (e.g., nutrients) to optimize plant and microbial breakdown of oil under recalcitrant conditions. Additional funding has been made available by BP for applied research on oil spill dynamics and outcomes. Managed by the Gulf of México Research Initiative (GoMRI), this 10 year \$500 million program aims to advance comprehensive knowledge of oil spills. The program will undoubtedly offer stronger platforms for innovation in oil spill response, including improved methods for shoreline remediation<sup>7</sup>.

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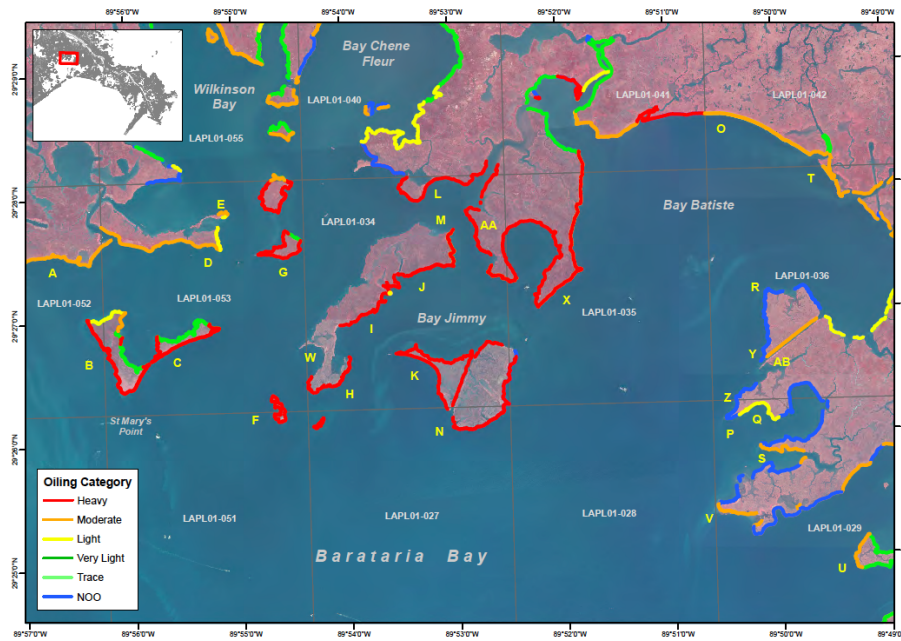
<sup>7</sup> <http://gulfresearchinitiative.org/about-gomri/gri-mission>

### 3.7 Proving Grounds for Shoreline Remediation and Restoration

Bay Jimmy has become a testing ground for determining how to improve the process and outcomes of shoreline remediation, with the goal of identifying clear pathways toward recovery [34]. Remediating oil from coastal marshes presents technical (i.e. what approach will remove the greatest amount of oil while doing the least harm) and logistical (i.e. when and how rapidly should oil be removed) challenges that can involve considerable trade-offs. Without greater understanding of potential outcomes of alternative treatment approaches, decisions to escalate remediation to meet immediate demands could jeopardize long-term ecosystem recovery [59]. It is possible, for example, that reducing environmental exposures to oil may come at the expense of marsh integrity. Removing surface mousse<sup>8</sup> can reduce risks of immediate resuspension, while increasing the risk of subsurface deposits being released into marsh interiors with concomitant rises in plant mortality. Wholesale removal of soil, organic debris, and plant cover during treatment also can endanger marshes by reducing elevation (i.e. loss of above and below ground biomass and subsidence through elevated metabolism of subsurface organic soils following exposure to oxygen) [60-64]. Aggressive remediation that increases inundation and erosion can result in greater rates of marsh loss and conversion of shorelines to open water, especially in areas like Barataria Bay where background rates of loss are among the highest on the Gulf coast [65]. Alternative approaches that leave oil in marshes may not immediately eliminate risks of chronic exposure and toxicity, but marsh platforms remain largely intact as risks of exposure decline over time due to natural attenuation, burial and weathering.

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<sup>8</sup> Mousse refers to oil that has emulsified and become a suspension of oil and sea water



**Figure 3.7:** Map representing the distribution and intensity of oiling in Northeastern Barataria Bay, Louisiana. Shoreline was categorized and identified for remediation according to extent of oiling (red = heavy, yellow = moderate, green = slight). Shoreline “K” in Bay Jimmy is host to ongoing studies of shoreline remediation and recovery. Map courtesy of Scott Zengel, NOAA.

Recognizing that marsh habitat is difficult to regain once it is lost, the Unified Incident Command approved a plan for conducting tests in Northern Barataria Bay to evaluate outcomes of alternative treatment approaches. Led by Dr. Scott Zengel, the lead scientist overseeing shoreline assessment for NOAA, a remediation treatment study was implemented to improve decision-making for ongoing marsh cleanup efforts [34]. The study was designed to evaluate three primary treatments: vegetation cutting, vegetation raking, and vegetation raking followed by cutting; followed by four secondary treatment techniques: low-pressure flushing, two types of surface washing agents, and vacuuming. Comparisons were drawn to areas that received no treatment (i.e. areas set aside to undergo natural recovery), and unoiled areas that served as controls. On shoreline “K” of Bay Jimmy (Figure 3.7), treatments were randomly assigned across plots measuring 8.5 m along the water’s edge, and 10 m toward the marsh interior including the oiled wrack line bounding landward contamination. Unexposed control sites were located on nearby shorelines (Figure 3.7). Treatments were applied adaptively, allowing ineffective techniques to be discarded as tests proceeded. For example, vegetation cutting using a weed trimmer was immediately abandoned after various cutting attachments failed to remove oil mats, even after plots underwent preparatory raking. Instead, raking was used to break up oiled mats until the mousse below was exposed to weathering.



Early observations revealed no difference in oil characterization following the initial treatment tests: vegetation left standing had subsequently laid down, trapping oil beneath a new tarry mat. New tests employed more aggressive techniques, including raking down through the oil and cutting vegetation with articulating hedge trimmers on poles (Figure 3.8). Mousse was raked onto standing vegetation, allowing it to be cut out and removed with exposed plant growth. Raking and cutting were alternated until only clean sediment remained [34]. Responders gathered oiled debris for disposal, then proceeded to test secondary treatment options. Low-pressure washing and each of two surface washing agents (Cytosol and PES-51, both NCP Product Schedule listed<sup>9</sup>) were tested in three different plots. These treatments resulted in scouring upon application and failed to release oil from the marsh beyond sheening. Vacuuming from marsh boards resulted in the recovery of more water and sediment than oil, and also promoted subsurface penetration of oil. Further tests of secondary treatment techniques were subsequently canceled [34].



**Figure 3.8:** Responders hand raking mousse oil, Shoreline “K”, Bay Jimmy, Louisiana. Image courtesy of PJ Hahn.

Conditions in the treatment and control plots were monitored on a monthly basis from October 2010 through September 2011 following SCAT protocols to characterize oil, sediment chemistry, vegetation cover, and benthic macroinvertebrates. SCAT assessments involve measuring oiling distribution (length, width, percent cover); oiling type (oiled wrack, oiled vegetation/debris mats, oil on standing vegetation, oil on/in substrate); oil thickness; and oil character (liquid oil, mousse, surface residue, tar, etc.). Cross-sections from dominant oiling

<sup>9</sup> <http://www.epa.gov/oem/content/ncp>

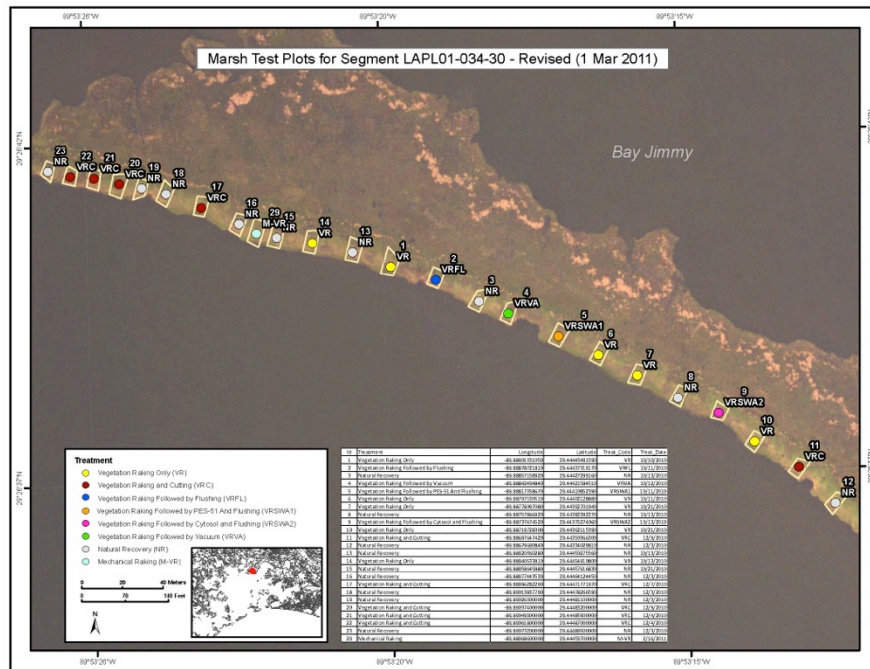
zones were used to quantify oil burial, penetration, and mixing into subsurface sediments.

Preliminary findings of the remediation study indicated that aggressive treatment enabled effective recovery of oil without jeopardizing marsh integrity [34]. Aggressive raking and cutting was the only treatment that completely and persistently removed oiled vegetation mats, and that left no evidence of increasing oil penetration or mixing in subsurface sediments. Vegetation regrowth appeared to be greater in aggressively treated plots than other treatment plots, which in turn experienced greater regrowth than plots undergoing natural recovery. Preliminary surface sediment chemistry data, however, indicated that total petroleum hydrocarbons and polycyclic aromatic hydrocarbon content did not differ across the treatments, though levels were slightly lower and more weathered in aggressively treated plots [34].

Although the treatment study represents an innovative effort to provide standardized, replicated comparisons of treatment options to inform ongoing remediation efforts, follow on studies could provide more rigorous understanding of post-remediation shoreline recovery [15, 66]. Coastal marsh responses to disturbance can span years to decades. Plant responses, soil oxidation, rates of decomposition, and consequences of compaction, subsidence, and erosion are all important ecosystem characteristics that have not yet been properly assessed. Continued monitoring of the treatment plots represents a singular opportunity to track ecosystem recovery following alternative shoreline remediation approaches [30]. It is unclear, however, whether the treatment plots will remain available for study because completion of Stage III response requires that all oiled shoreline receive treatment to satisfy NFT guidelines. Petitions have been filed to exempt the study plots from treatment, highlighting how useful continued research would be for future oil spill responses [34].

Bay Jimmy has also become a testing ground for achieving better integration of remediation and restoration. Undertaking restoration alongside remediation—something that could be referred to as “restorative remediation”—can enhance treatment and recovery of sensitive ecosystems including coastal marshes in erosional environments [67]. Under the current plan, restoration is not part of the NCP and NIMS. Barring a settlement, restoration follows completion of the NRDA process - after data is gathered to determine resource injury, after economic and scientific studies are conducted, after a restoration plan is developed, and after trustees identify restoration projects of interest [68]. Consequently, years can pass between shoreline remediation and restoration. Long delays between remediation and restoration elevate risks of habitat loss and resulting losses of dependent species and valued ecosystem services [67], especially in erosional environments like Bay Jimmy. Accordingly, restorative remediation (as compared to emergency restoration or restoration following the NRDA process) can potentially reduce responsible party costs and long-term natural resource damages. Restorative remediation might also reduce costs by eliminating redundant logistical expenses.

Restoration could be readily implemented via response personnel and equipment marshaled for shoreline treatment. Denuded shorelines in Bay Jimmy, for example, could have been anchored with plants to replace lost vegetation after remediation crews removed oiled material. Although concerns about rates of survivorship of transplants in oiled sediments must be addressed, integrating restoration with remediation could immediately address concerns of loss while making best use of available resources [67].



**Figure 3.9:** Marsh test plots, Bay Jimmy, Louisiana. Restoration study treatments are randomized alongside the NOAA remediation treatment plots.

The test plots in Bay Jimmy were been planted with arrays of native genotypes, Vermilion, and other cultivar genotypes to first assess how planting contributes to the recovery of remediated shoreline and to also assess how use of different parent stocks can influence ecosystem attributes. For each plot, bare-root stems were hand-planted in four rows perpendicular to the shoreline, spaced on 1 meter centers (Figure 3.10). Plants began 5 m from the water’s edge, and each row contained 11 stems spaced 0.5 m apart. Baseline characteristics of soil structure and content, surface and subsurface hydrocarbon content, and plant productivity were measured prior to planting. Plot characteristics have subsequently been monitored on a monthly basis, with additional information on accretion rates, soil stabilization and soil development collected at quarterly intervals. By capturing regular and stochastic disturbances, such as storm events, the study offers opportunities to assess shoreline resilience [69].



**Figure 3.10:** Shoreline restoration studies conducted in Bay Jimmy; transplant plot (left); propagation tube plot (center); detail of propagation tube (right).

Smooth cordgrass functions as an “ecosystem engineer” by regulating physical and biological conditions independently of the local environment [67]. The addition of smooth cordgrass to remediated shoreline can prevent marsh loss by trapping mineral sediment, adding organic biomass to substrates, and by armoring platforms against tidal erosion. Replanting shorelines may also encourage oil degradation by oxygenating soils, elevating microbial metabolism in soils, and uptake of hydrocarbons from soils [70]. Different smooth cordgrass genotypes, however, exhibit variation in functional performance [70]. Properties known to vary according to *S. alterniflora* genotype range from plant community composition, microbial activity and diversity, organic matter distribution, and the presence of fish larvae in the marsh [15, 71-74]. Marsh restoration projects in Louisiana nonetheless are now required to use a single smooth cordgrass genotype, referred to as Vermilion, which has been cultivated for maximum aboveground biomass, disease resistance, and transplantation survival at the expense of other traits such as decreased belowground biomass [74, 75]. Use of cultivars for marsh restoration can alter local gene pools through replacement or admixture with native genotypes, and therefore by extension, conventional restoration can result in unexpected and potentially undesirable ecosystem properties.

Improving restoration technologies to decrease the labor, expense, and risk associated with planting marsh vegetation could further promote recovery of remediated shorelines. Because smooth cordgrass exhibits low seed fecundity (viability), restoration projects often involve manual installation of plants. Using stems, plugs, or containers costs an average of \$9,000 per acre in Louisiana CWPPRA projects and requires labor ranging from 25 to 125 hours per acre [65, 76]. Besides the costs involved, logistical challenges of manual installation limit the feasibility of large-scale implementation. Salt marshes are often remote environments that are difficult to access. Also, marsh substrates are fragile, so entry and movement within a marsh can result in considerable damage.

Members of the academic-industry-agency partnership undertaking transplant studies in Bay Jimmy are also testing prefabricated technology for shoreline restoration. Biodegradable mesh tubes have been designed and built to contain

smooth cordgrass rootstock in a bagasse<sup>10</sup> growth medium (Figure 3.10). The product design enables plants to be introduced to targeted restoration sites by simply laying out and securing 'propagation tubes' on exposed shoreline. Incorporation of plants into the design allows natural root growth to help anchor tubes securely to the marsh. The tubes therefore promote regrowth while armoring shorelines against erosion.

During experimental trials conducted in Bay Jimmy, tubes were established in plots measuring 15 m wide along the shore, and 15 m long from shore. The propagation tubes were initially arranged as a comb with four tubes perpendicular to the shoreline (spaced one meter apart) abutting a fifth tube that was placed on top of the shoreline scarp. The tubes were secured with wooden furring strips at 1 m intervals. This arrangement proved unstable, however, during storm events. In subsequent trials the comb arrangement faced the water, which minimized stress from wave impact. Also, the interior tube trapped debris carried to shore, resulting in the rapid development of organic wrack. Other preliminary observations indicate that the propagation tubes are a promising tool for decreasing marsh restoration labor and expense, while increasing the pace of shoreline development and facilitating lateral growth of the marsh surface. Smooth cordgrass root masses in deployed tubes, for example, have exhibited nearly 100% survivorship. The slow deterioration of the tubes and expansion of root masses also appears to be enabling plants to become firmly embedded in the marsh platform. Further monitoring and additional trials will be necessary to quantify rates of regrowth, shoreline development, and marsh accretion [67].



**Figure 3.11:** Smooth cordgrass root mass (left); bagasse-filled mesh tube (right). Image courtesy of Allyse Ferrara, Nicholls State University.

### 3.8 Planning for the Remediation and Restoration of Oiled Shorelines

The Macondo well blow out resulted in an environmental disaster of global proportions. In an era of energy production shifting away from coastlines, it has redefined our understanding of risks associated with deep water wells. It has enhanced our awareness of the intricate complexity of communities whose

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<sup>10</sup> Bagasse is a waste product left over from refining sugarcane that is readily available from the Louisiana sugarcane industry. Bagasse diverted from processing plants can be supplemented with organic substrate to facilitate plant establishment.

livelihoods rely as much on the energy sector as on fisheries that are at risk from well blowouts. It has also refocused our attention on Gulf coast ecosystems, including at-risk areas of the Mississippi River Delta that sustain ecological and cultural resources of national importance.

Understanding of ecological and related economic outcomes of the DWH oil spill remains cursory, including potential timelines of recovery (i.e. return to a state comparable to states exhibited by unimpacted sites). Based on commonly measured ecological parameters (e.g. vegetative cover and structure, species diversity, petroleum hydrocarbon concentrations in soils), recovery times for oiled marshes can range from a few weeks to decades. Recovery times spanning from many years to decades have been documented for marshes in cold temperate environments, in sheltered locations, that were heavily oiled with fuel oils such as bunker C or no. 2 fuel, and that were damaged by intensive remediation methods [67]. Under recalcitrant conditions, oil persisting in buried sediments can continue to influence the integrity of coastal ecosystems long after a spill [12]. Four decades after the 1969 *Florida* barge spill in Wild Harbor (Massachusetts), oil remaining in marsh sediments continued to stunt below-ground growth, with affected areas exhibiting lower marsh elevations and greater bank erosion [77-79]. Long recovery times were also found following a spill in Buzzards Bay, Massachusetts; the *Miguasha* spill in Canada; the *Metula* in Chile; and the *Amoco Cadiz* in France [66]. Recovery times of less than a year were found for marshes in warm climates that experienced light to moderate oiling with light crude oil and little or no remediation [79-82]. Several of the spills resulting in short recovery times have occurred in Galveston Bay and other areas of Texas [12]. Similar recovery rates might be expected following the DWH spill (i.e. evidence of natural recolonization and regrowth has been found in some oiled marshes), except that oil from the blown Macondo well grounded on to erosional shorelines and heavily degraded deltaic wetlands that are hotspots of habitat loss. Aggressive remediation (e.g. destroying the marsh to save it by stripping sediment and plants) could also compound delays or fully prevent recovery, given the possibility of accelerated habitat loss [12].

Redressing shoreline damage from the DWH event requires science-based approaches that address the trifecta of oiling, erosion and subsidence. Although it is perhaps too late now, embracing a policy of shoreline remediation followed by habitat restoration can promote post-spill recovery while preventing habitat loss from erosion or subsidence. Restoration should not be considered a consequent step to remediation, but rather an important remediation technology in its own right, imperative to protecting oiled shoreline from damage and loss. The potential for restoration to promote post-spill recovery through revegetation or accelerating natural recolonization has been widely recognized [67, 81, 82]. Baker, for example, suggested that faster recovery of marshes might be achieved by planting *Spartina* shoots directly into oil laden sediments [83]. Lin and Mendelsohn [84] have shown that *S. alterniflora* can successfully recolonize areas with oil concentrations as high as 250 mg g<sup>-1</sup> so long as the oil was sufficiently weathered. Although little formal work has been done to assess post-spill restoration outcomes, Bergen et al. [67]

found that replanting significantly improved marsh recovery after the 1990 *Arthur Kill* oil spill in New Jersey [84]. Oiled salt marshes where smooth cordgrass was replanted exhibited 70% vegetative cover after 3 years, whereas only 5% coverage was achieved at oiled sites that were not re-planted [67]. The treatment study and follow-on restoration studies in Bay Jimmy represent important steps towards achieving similar outcomes for Gulf coast marshes.

Restoring oiled shorelines to conditions comparable to natural ecosystems is a deceptively simple goal. Conventional restoration practices often fail to recover original levels of ecosystem function and structure [85]. Understanding the ecological consequences of practical trade-offs can help minimize undesirable outcomes. Some choices made during project execution, as simple as the spacing of transplanted propagules, can lead to failure. Other choices, such as replanting shorelines with ecosystem engineers (e.g. smooth cordgrass) can modify ecosystem attributes and result in alternative states that will never resemble reference conditions [85]. Although conventional practices can serve as precautionary measures to ward off the specter of habitat loss, innovative methods for shoreline restoration may prove critical for the recovery of Gulf coast ecosystems.

Shoreline remediation and restoration should be guided by comprehensive coastal restoration plans. It has long been recognized that coastal ecosystems of the northern Gulf of México, and in particular wetlands of the Mississippi River Delta, are in dire need of restoration. Vast areas of the Mississippi River Delta are being lost and will continue to disappear without restoration being undertaken at a grand scale. Many of the challenges of coastal restoration are well-recognized and are being addressed in regional and state-wide plans [86] that have broad support from coastal scientists and stakeholders. These plans can therefore serve as a secure platform for remediation and restoration of oiled shoreline. New challenges could surface, however, as information becomes available from ongoing studies of coastal ecosystem responses to oiling. Accordingly, greater reciprocity between oil spill response efforts and coastal restoration planning will help ensure that progressive measures are taken to secure the future of Gulf coastal ecosystems.

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## Chapter 4

### Bioremediation of Hydrocarbons: An In-situ approach to Remediation of Oil in the Soil Environment

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#### 4.1 Introduction

The National Incident Command estimated that 4.9 million barrels of oil were released from the BP Deepwater Horizon event [1]. Due to the limitations of current oil spill response technology, only a portion of the oil was recovered before reaching shorelines and sediments [2]. With regard to the oil released during the BP incident, a siphon, inserted at the leaking wellhead, captured 17% before it entered the Gulf, mechanical skimming efforts recovered 3%, in-situ burning distributed 5% into the atmosphere, and the application of chemical dispersants displaced 16% from the water's surface [3, 4]. More than 2.89 million barrels remain in the Gulf ecosystem, much of which will eventually reach a shoreline, where its fate will be determined by a number of environmental factors [5-7]. The ability to remove the oil that reaches sensitive shoreline ecosystems is rather limited<sup>1</sup>. This chapter explores an on-going research project focused on developing remediation technology and tools for treating oil-contaminated soils. The remediation approach adopted by this project is closely modeled after processes that occur naturally.

Natural attenuation refers to the process resulting from naturally-caused biological, chemical, and physical reductions of the mass or concentration of a contaminant in the environment [8-10]. Under favorable conditions and over an extended period of time, contaminants may reduce in mass, toxicity, mobility, volume, or concentration, thus diminishing their potential harm [11]. For petroleum hydrocarbons in soil, these reductions occur through a number of mechanisms, such as: biodegradation, volatilization, and dispersion, facilitated by organisms [11]. Biodegradation is the transformation of hazardous organic materials to non-hazardous compounds through the biological actions of microorganisms [11-13]. The aerobic breakdown or decomposition of hydrocarbons results in the simpler molecules, carbon dioxide and water [11]. Most soils contain a large number of naturally occurring microorganisms, including bacteria, fungi, protozoa, and algae [14]. Heterotrophic bacteria are dependent on organic substances for nutrition. These bacteria will metabolically oxidize carbon to produce energy, converting it to carbon dioxide [15]; their ability to utilize organic compounds, such as plant debris and petroleum constituents, allows for the bioremediation of the latter [12].

However, natural attenuation rarely results in the complete removal of polyaromatic hydrocarbons (PAHs) from the soil [8]. Often, contaminants remain

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<sup>1</sup> <http://www.itopf.com/spill-response/clean-up-and-response/shoreline-clean-up>

because they are bound to particles such that they are no longer bioavailable, inhibiting further degradation [11, 16]. To help facilitate remediation in soil, biological treatments, such as landfarming and composting, have been employed at oil-contaminated sites [17-21]. Landfarming works by stimulating aerobic microbial activity within soils through aeration and the addition of nutrients and moisture [22]. These treatments have limitations and new research is focused on overcoming challenges such as remediating soil-bound hydrocarbons and nonvolatile PAH residues that are recalcitrant to biodegradation [11, 23, 24].

## **4.2 Research Background**

My early graduate research focused on conventional agriculture practices, which rely on the application of synthetic chemicals in order to enhance soil fertility and crop pathogen resistance [25-27]. Implicit in the conventional agricultural approach is the assumption that plants are static and defenseless organisms requiring manufactured chemicals in order to deliver viable agroproduce. Although plants in the agricultural context are part of a constructed ecological system, the system still operates under many of the same rules as natural ecosystems [28]. In an agricultural ecosystem, crop health and productivity can be correlated with the relationships between the community members of such a system [29, 30]. Natural ecosystems tend to increase in stability in direct proportion to the robustness of their diversity—a more diverse ecosystem is more robust [31]. Robustness, in this context, can be defined as an indication of the overall health or resilience of the ecosystem as it relates to a plant's ability to recover from disturbance. In other words, diversity can help protect primary producers (plants) against perturbations, such as disturbances from agricultural pests and pathogens [32-34].

The conventional agriculture model posits that, by sterilizing soil (e.g. methyl bromide or chloropicrin treatment) and by adding quantities of specific fertilizers, a predictable volume of produce (product) will be reliably generated [35, 36]. However, changes in pest resistance to pesticides and increased soil erosion interfere with this model [30, 33]. Therefore, crop productivity cannot be determined from the application rate of synthetic chemical inputs only, but should also consider factors related to a crop ecosystem's ability to suppress pests, pathogens, and the soil erosion conditions [32]. A crop ecosystem that supports and encourages strong relationships between mutually supportive members in a soil community is less likely to allow the introduction and settlement of plant pathogens, and is more likely to support healthy soil conditions [25, 28].

Agroecology is a strategy for farming which challenges the conventional view of crop productivity and instead emphasizes providing support for the natural ecological processes, such as nutrient cycling, predator/prey interactions, competition, and successional changes [28, 29]. For example, the addition of compost and vermicompost promotes healthy soil within the agricultural system

[37, 38]. Composting relies on natural and controllable processes and contributes soil nutrients and beneficial microbes. Composting also allows the use of local biodegradable material, that would otherwise end up in a landfill, to be repurposed to support food production [38, 39]. The microbial flora delivered through vermicompost products enhanced plant performance in terms of nutrient uptake and growth [40]. An appropriate combination of vermicompost products and synthetic fertilizers accelerated plant development and required less fertilizer input. Considering the several ways in which earthworms and compost have been used to enhance crop productivity and resistance, and considering how little we know about them in an agricultural context, I designed and implemented a research experiment to help further describe the advantages and challenges of earthworm culture.

On November 7, 2007 a cargo ship struck a structural support on the Bay Bridge, breaching the hull and releasing 202,781 liters of intermediate fuel oil <sup>2</sup> (bunker C) into San Francisco Bay. Bunker C, or No. 6 fuel oil, is a dense, highly viscous oil with an approximate 14 API gravity<sup>3</sup> [41]. Bunker C oil is created by blending heavy residual oil (No. 6 fuel oil) with lighter oil (No. 2 fuel oil) [41]. As a result, only about 5-10 % will evaporate within the first hours of a spill and the heavy No. 6 oil will separate to form patches or tarballs [42]. No. 6 fuel oil can coat wildlife dwelling on the surface water, smother intertidal organisms, eliminate marsh vegetation by coating plants, pose a high mortality rate for seabirds, waterfowl, and fur-bearing marine mammals, sink to form tarmats or tarballs, and/or may be carried hundreds of miles by ocean currents [43-45].



**Figure 4.1:** Presidio remediation project. Adding oil-contaminated hair mats to green-waste compost for the start of the bioremediation process (left). Distributing the hair mats in preparation for thermophilic composting (right).

I designed and conducted research on fuel oil in the Presidio National Park, after a failed mycoremediation experiment, in an effort to test bioremediation techniques

<sup>2</sup> [http://www.dfg.ca.gov/ospr/NRDA/cosco\\_busan\\_spill.aspx](http://www.dfg.ca.gov/ospr/NRDA/cosco_busan_spill.aspx)

<sup>3</sup> [http://www.etc-cte.ec.gc.ca/databases/oilproperties/pdf/web\\_bunker\\_c\\_fuel\\_oil.pdf](http://www.etc-cte.ec.gc.ca/databases/oilproperties/pdf/web_bunker_c_fuel_oil.pdf)



for oil collected with organic fibers during cleanup. The remediation treatment incorporated composting, microbial inoculation, and vermicomposting with *Eisenia fetida* earthworms (Figure 4.1). The remediation project demonstrated the potential for this technology to reduce total petroleum hydrocarbon (TPH) contamination effectively in soils [17, 18, 46-51].

Table 4.1 shows that there was a significant reduction in the concentrations of Motor and bunker C oil from the beginning of the project to the end of the vermicomposting phase. There was as much as a 96.5% reduction in total petroleum hydrocarbons (TPH). These values represent the concentration of PAHs on the hair mats and the dilution due to the addition of organic matter, then analyzed for total extractable petroleum hydrocarbons. Earthworms remained active after processing the composted green waste and oil mixture. In fact, earthworms were found concentrated around the decomposing oil hair mats, possibly due to the presence of high moisture and microbial activity. These observations demonstrated that composting could reduce contamination levels below the ecotoxic levels for earthworms and other macroscopic living organisms [52, 53]. The odor of oil was absent upon finishing vermicomposting, also indicating that earthworm stimulated aerobic microbial activity had effectively degraded a considerable amount of the oil.

START		FINISH	
<b>Motor Oil</b>	<b>mg/kg</b>	<b>Motor Oil</b>	<b>mg/kg</b>
MOB1	22,000	VC1	23
MOB2	2,300		
MOB3	19,000	<b>Bunker C Oil</b>	<b>mg/kg</b>
MOB4	2,300	VC1	28
<b>Bunker C Oil</b>	<b>mg/kg</b>		
BB1	860		
BB2	1,800		
BB3	780		
BB4	120		
<b>Total</b>	49,160		
<b>Average</b>	6,145		
<b>Median</b>	2,050		

Key	
MOB	= motor oil barrel
BB	= bunker c barrel
VC	= vermicompost

**Table 4.1:** Presidio remediation total extractable hydrocarbon analysis prior to (left) and after (right) vermiremediation treatment

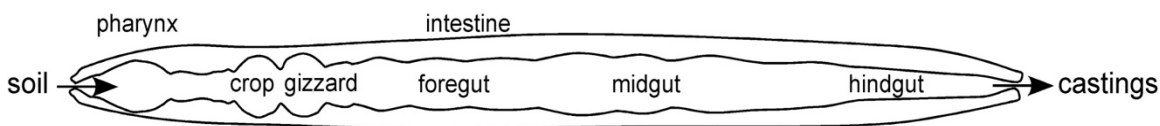
The positive results of this initial study led to a funding opportunity to conduct more detailed experiments to understand better the factors influencing the outcome

of PAH degradation and the mechanisms contributing to their transformation [16]. Chevron Energy Technology Company created a program to develop remediation methodologies and technologies that reduce remediation costs<sup>4</sup>. Chevron's environmental liability includes heavy hydrocarbons such as weathered crude oil and PAHs impacted by upstream activities (e.g. oil field exploration and production) and downstream (e.g. refining, bulk storage and transfer). A request for proposals was issued by Chevron for the development of new remedial methodologies and technologies that cost-effectively reduce environmental impacts. I submitted a proposal and received a grant with the objective of better understanding the chemical, biological, and physical contaminated-media interactions for the purpose of facilitating bioremediation of PAH contaminated soil.

### 4.3 Vermiremediation Technology

Attenuation of PAHs can be stimulated by creating near-optimal conditions for the activity of microbial species with the capacity to degrade hydrocarbons [54, 55]. Earthworms can contribute to attenuation by aerating soil, excreting castings (rich in enzymatic proteins), and increasing the bioavailability of nutrients (nitrogen, potassium) [48, 56-58]. Studies to date suggest that hazardous components of petroleum products, including PAHs can be degraded by earthworms in both amended soils [59-61] and during vermicomposting [62]. Vermiremediation is the use of worms to stimulate contaminant degradation or sequestration from soils [63].

Vermiremediation is particularly well suited to water-unsaturated soils (such as vadose zone soils and previously excavated soils), because these conditions facilitate the introduction of organic materials as feed stocks for earthworms, which encourage the burrowing and feeding behaviors that mix and aerate the medium [64].



**Figure 4.2:** Earthworm bioreactor (gut). Image courtesy of Clive Edwards, Ohio State University.

Earthworms ingest and process large quantities of soil, exposing them to organic contaminants that may be degraded during transit through the earthworm gut (Figure 4.2). The mechanisms of vermiremediation are not fully understood, but it appears that it leads to the enhancement of PAH degradation by soil mixing and stimulation of microbial activity by earthworms [56]. Both of these processes may

<sup>4</sup> Chevron 2010 RFP Remediation of Heavy Hydrocarbons and PAHs in Impacted Vadose Zone Soils

contribute to release of entrapped and sorbed contaminants thus increasing the availability of the PAHs to microbes [65, 66]. As an additional benefit, earthworms improve soil nutrition, increasing options for soil use after remediation of the site [67].

There are conflicting reports for mechanisms of PAH removal by the worms in the literature—degradation [60] or sequestration [68, 69]. Understanding mechanisms of PAH reduction will enable design of more effective vermiremediation methods. The feasibility of vermiremediation of PAHs was previously tested in my Presidio fuel oil experiment, but more analysis is required to understand its potential as a remediation technology [48, 49, 57]. Vermiremediation as a strategy for PAH impacted soils created the basis for the research presented in this chapter.

## **4.4 Research Projects**

Vermicompost tea (vermitea) is “brewed” from worm castings, water, supplemental food sources, and the addition of air to promote development of aerobic microorganisms [70]. The castings contain trace minerals, micronutrients, plant growth hormones (cytokinins, auxins, and gibberellins), and amino acids [64, 71]. Microbial food sources used for brewing include kelp, humic acid, and fish hydrolysate to promote bacterial and fungal development during the 24-hour brewing period.

The purpose of these projects is to understand the effect of vermitea on the remediation process of hydrocarbon-impacted soils. The effectiveness of the application of vermitea on weathered crude oil soils will be examined through treatment and control samples, as well as through the analysis of the microbial population demographics. Vermitea was analyzed for the presence of biosurfactants and shifts in microbial communities, with an emphasis on hydrocarbon-degrading taxa. Biosurfactants are produced by microorganisms to increase the surface area and bioavailability of hydrophobic water-insoluble substrates, such as petroleum hydrocarbons [72].

### **4.4.1. Microbial Community Analysis**

#### **Objectives**

Earthworms ingest soil microbial populations that change with transit through the gut [73, 74]. Some are stimulated while others are eliminated prior to exiting the gut as castings, directly impacting the soil microbial community [74]. Ingested protozoans are digested in the crop, gizzard, and foregut, serving as a food source to the worms [75]. The microbial community in an earthworm’s gut, called the “gut microzone”, likely changes composition in response to crude oil, but this has not

been well-documented, and only one study examining the total microbial phospholipid fatty acid profiles was able to detect a clear shift [76]. Survival of some earthworms at higher exposure concentrations might be explained by successful adaptation of the microbial communities in the gut microzone of these individuals [73, 74].

Preliminary experiments analyzed the change in earthworm microbial communities in response to the worms feeding on oil-contaminated soils, thus helping to inform potential remediation strategies. By identifying key taxa of the hydrocarbon-degrading community, the vermiremediation technology can be enhanced through earthworm feedstock and selective breeding to improve PAH tolerance [18, 54, 55].

## **Methods**

The Phylochip (microbial array) analysis was performed by Thomas Azwell, Felicia Chiang (UCB), and Lauren Tom (LBNL), using a control and treatment vermitea sample, in triplicate. Alaska North Slope (ANS) crude oil was used to stimulate a microbial response for the initial experiments. Future experiments will employ San Joaquín Valley (SJV) crude oil, which is more viscous and more similar to a weathered crude oil found in contaminated soils.

The Phylochip instrument was selected for the microbial analysis of the vermitea because of its ability to identify multiple bacterial species and organisms from complex microbial samples [77]. Phylochip data are generated through the detection and comparison of the 16s RNA bacterial gene in samples with a known bacterial gene library [77]. This process allows for a comparison of the proportion of bacterial phyla in the tea with and without oil and the abundance of phyla known to produce surfactant compounds and break down hydrocarbons [78].

I performed this test, with the help of my research assistant, Felicia Chiang, by first collecting the messenger RNA molecules present in the microbial population of the vermitea [79]. Each mRNA molecule is then labeled by using a reverse transcriptase (RT) enzyme that generates a complementary cDNA to the mRNA [80]. The cDNAs are placed onto a DNA microarray slide and the labeled cDNAs that represent mRNAs in the cell will then bind to their synthetic complementary DNAs attached on the microarray slide [81].

## Materials and Protocol

### Vermitea Brewing Method

#### Materials:

- 0.2 g humic acid<sup>5</sup>
- 1.2 mL kelp fertilizer<sup>6</sup>
- 1.2 mL fish hydrolyzate<sup>7</sup>
- 20 mL worm castings ( $\approx$ 20 grams)
- 1000 mL deionized H<sub>2</sub>O
- 2000+ mL beaker
- 500 mL separatory funnel



**Figure 4.3:** Bench-scale vermitea brewing system using 500 mL separatory funnels.

#### Protocol:

1. Fill large beaker with deionized H<sub>2</sub>O and add the kelp fertilizer, fish hydrolyzate and humic acid.
2. Put worm castings in a 400-micron polyester multifilament mesh filter bag (PEMU).
3. Squeeze out castings with bag immersed in the beaker for 2 minutes.
4. After 2 minutes the remaining material left in the bag will be minerals and solid chunks of organic matter.
5. Add the water and extract to the separatory funnel and run aeration system for 24-hours.

### Contamination of Soil Samples

#### Materials:

- Alaska North Slope (ANS) crude oil, provided by Chevron Corporation, Richmond, California
- Dichloromethane (DCM)— Sigma-Aldrich, St. Louis, Missouri
- PAH contaminated soil, provided by Chevron Corporation, Port Arthur, Texas (84.083 % dry weight)
- oven

#### Protocol:

1. Weigh out approximately 800 grams of soil.

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<sup>5</sup> Down to Earth, Eugene, Oregon. Humplex contains 50% inert ingredients and 50% Leonardite humic acid (LHA). The elemental composition of LHA is 63.81% C, 3.70% H, 31.27% O, 1.23% N, 0.76% S, and <0.01% P by weight, on an ash-free and moisture-free basis. The ash content is 2.58% by weight. All data reported by the International Humic Substances Society (IHSS), <http://www.humicsubstances.org>.

<sup>6</sup> Sanctuary Blend, Monterey Bay, California

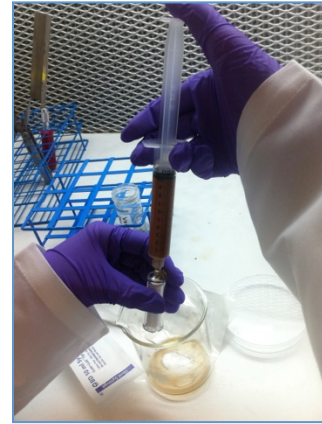
<sup>7</sup> Earthfort, Corvallis, Oregon

2. Weigh out 2 % (by dry weight) of the soil weight for the SJV crude oil.
3. Lower the viscosity of the ANS crude with dichloromethane by adding 15 % of the weight of the SJV.
4. Mix soil with oil with drum mixer for 24 hours.
5. Place soil in laboratory fume hood for 48 hours to vent.
6. Set vented soil into oven at 50 °C for 24 hours

### Extraction of Vermitea Samples

#### Materials:

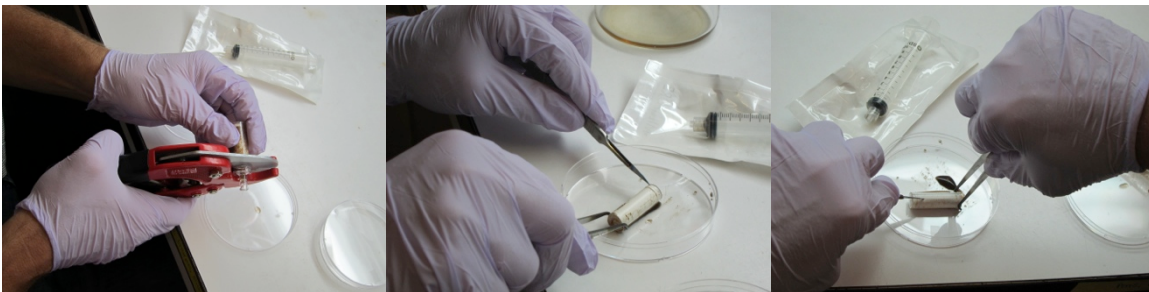
- 0.22 µm Millipore Syringe Filter Unit
- 10 mL Luer-Lok Tip Syringe



**Figure 4.4:** Luer-Lok syringe used for filtering vermitea

#### Protocol: Filter Preparation

1. Fill syringe with 10mL of vermitea sample.
2. Attach filter and inject 10mL of vermitea, reset and inject 10mL of air.
3. Use cutting tool to remove distal end of filter (see Figure 4.5).
4. Place filter roll on petri dish and cut to completely remove filter.
5. Place center section, representing approximately 1/2 of filter, in sample tubes and follow DNA extraction protocol (see below).



**Figure 4.5:** PVC pipe cutter, scalpel, and forceps used to remove filter roll.

#### Materials:

- Miller phosphate buffer: 100 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 8.0
- Miller SDS buffer: 100 mM NaCl (from solid) 500 mM Tris, pH 8.0 (from 1 M solution) 10 % SDS, wt./vol. (from 20 % solution)
- Phenol:Chloroform:Isoamyl alcohol (25:24:1)
- Chloroform
- MO BIO Laboratories, Inc. (MoBio) Solutions: S3 (12800-50-3), S4 (12800-50-4), S5 (12800-50-5)
- MoBio Spin Filters
- High-pressure homogenizer (e.g. Bio 101 Fast Prep 120)

### Protocol: gDNA Extraction<sup>8</sup>

1. Add 300  $\mu$ L Miller phosphate buffer and 300  $\mu$ L Miller SDS lysis buffer.
2. Add 600  $\mu$ L Phenol:Chloroform:Isoamly alcohol.
3. Shake tubes with a high-pressure homogenizer for 45 sec at a speed of 5.5 m/s.
4. Centrifuge tubes at 10,000 x g for 5 min at 4 °C.
5. Transfer ~400  $\mu$ L of the aqueous supernatant solution to a phase-lock gel tube.
6. Add 1x volume of chloroform, mix by inversion for 5 sec.
7. Centrifuge tubes at 10,000 x g for 5 min at 4 °C.
8. Transfer approximately 350  $\mu$ L of the top layer into a new 2 mL tube. Record volume transferred. Transfer absolutely no chloroform at this step.
9. Add 2x volume of MoBio solution S3 to tube.
10. Mix by inverting five times and flicking gently (to prevent shearing of long DNA molecules).
11. Load MoBio Spin Filter with a 650 $\mu$ L aliquot and centrifuge 10,000 x g for 30 sec. Discard the flow-through and repeat with remainder of mixture.
12. Add 400  $\mu$ L Solution 4 to the filter and centrifuge 10,000 x g for 30 sec.
13. Discard the flow-through and centrifuge again at 10,000 x g for 1 min.
14. Carefully place spin filter in a new clean 2.0 mL tube. Avoid splashing any Solution S4 onto the spin filter.
15. Use a cellulose-fiber laboratory wipe to blot any liquid from the lip of the filter cartridge. (S4 can prevent DNA elution from the filter).
16. Carefully add 30  $\mu$ L Mo-Bio Solution S5 (Tris, pH 8.0) to the center of the white filter membrane. Let sit for 1 min.
17. Centrifuge 30 sec at 10,000 x g to elute the DNA.

### PCR Amplification for Phylochip Preparation

Prior to PCR amplification, genomic DNA should be quantified by fluorometer<sup>9</sup> using 2 ng of template in order to determine sample DNA concentrations [83]. For the PCR setup, all samples were diluted to 2 ng/ $\mu$ L and 1  $\mu$ L was used as a Template for PCR.

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<sup>8</sup> Miller DN, Bryant JE, Madsen EL, Ghiorse WC. (1999). Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Appl Environ Microbiol* 65: 4715–4724.

<sup>9</sup> Qubit fluorometer with dsDNA HS Assay Kit, Invitrogen (cat #Q3285)

**Table 4.2:** Initial PCR setup with TaKaRa ExTaq<sup>10</sup>

<b>Component</b>	<b>Volume per reaction</b>	<b>Final concentration</b>
Buffer w/ MgCl <sub>2</sub>	2.5 µL	1x
Forward primer (27F) <sup>11</sup>	1.67 µL	200 nM
Reverse primer (1492R) <sup>12</sup>	1.67 µL	200 nM
BSA <sup>13</sup>	1.25 µL	1 µg/ µL
dNTP mix	2 µL	200 µM each
ExTaq	0.125 µL	0.025 U
Template	1 µL	2 ng
Water	14.79 µL	
Volume per tube	25 µL	

**Amplification Protocol:**

1. Initialize<sup>14</sup> 95 °C 3 min.
  2. Denature<sup>15</sup> 95 °C 30 sec.
  3. Anneal<sup>16</sup> 50-56 °C 2 min.
  4. Extend<sup>17</sup> 72 °C 30 sec.
  5. Final Elongation<sup>18</sup> 72 °C 10 min.
- } 20 Cycles

Quantitation by Gel Electrophoresis

Initial PCR Test: 5 µL of each PCR sample and 15 µL of water were run on a 2% Agarose gel for 16 minutes at 60 V.

Gradient PCR setup: 4-temperature gradient with 4 tube replicates per sample (Same number of cycles, primer and template concentration used as the initial PCR test).

<sup>10</sup> DNA Polymerase (cat #RR001A), includes ExTaq, 10x Buffer, and dNTPs

<sup>11</sup> Primer Sequence: 27F (5'- AGAGTTTGATCCTGGCTCAG-3')

<sup>12</sup> Primer Sequence: 1492R (5'-GGTACCTTGTTACGACTT-3')

<sup>13</sup> BSA, Roche Applied Sciences (cat #10711454001)

<sup>14</sup> Heat activates the reaction

<sup>15</sup> Denatures the initial template into single-stranded DNA

<sup>16</sup> Anneals the primers to the single-stranded DNA template

<sup>17</sup> DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand

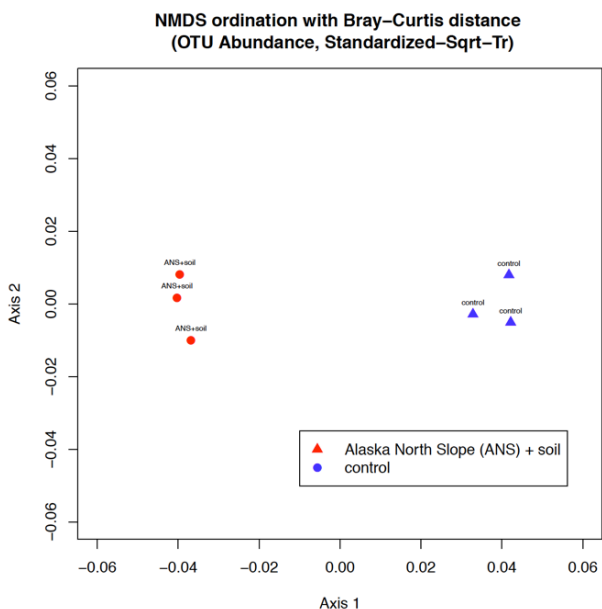
<sup>18</sup> Final incubation step promotes complete synthesis of all PCR products



The replicate tubes are combined, concentrated and purified for the chips. Gradient PCR is run as bacteria amplify differently at different temperatures, and having the gDNA replicates exposed to a gradient of temperatures reduces amplification bias.

## Results

Hydrocarbon-enhanced vermitea (Vermitea 2) was brewed as a preliminary assessment for changes in the microbial community and biosurfactants. Alaska North Slope (ANS) crude oil was used to stimulate a microbial response for initial experiments. The Phylochip analysis, completed by Lauren Tom (LBNL), revealed that many taxa increased in abundance in Vermitea 2 (PAH) compared to the control, Vermitea 1 (no PAH). In Figure 4.6, the non-metric multidimensional scaling (NMDS) is an ordination, or map of the samples where the physical distances between samples attempt to match the corresponding similarities in a community structure. The NMDS graph was generated by importing an untransformed Stage2 operational taxonomic unit (OTU) abundance report into Primer (version 6). The OTU abundance values were standardized and square root transformed within Primer-E and then exported as a .txt file into R statistical software (2.15.0).



**Figure 4.6:** Phylochip analysis of vermitea showing shifts in microbial communities. Data courtesy of Lauren Tom, Lawrence Berkeley National Laboratory.

The Bray-Curtis distance is commonly used in ecology as a measurement to express relationships between organisms [84]. The Bray-Curtis resemblance matrix is a triangular matrix of values where the values are Bray-Curtis coefficients calculated for every pair of samples [85, 86]. The Bray-Curtis coefficient ranges from 0 to 100 (100 when two samples are identical, and 0 when two samples have nothing in common). Triplicates of each vermitea sample were averaged then evaluated for fold-change differences. No averages were used to make the NMDS plot (Figure 4.6). Axis 1 and Axis 2 indicate 2-dimensional ordination space. In other words, the NMDS plot is a 2D representation of a many-D community structure.

Based on the Phylochip results, many taxa showed an increase in abundance in the crude oil enhanced tea in comparison to the control tea, including members of the Phyla *Bacteroidetes* (Order: *Flavobacteriales*, *Sphingobacteriales*), *Firmicutes* (Order: *Bacillales*), and *Proteobacteria* (Order: *Aeromonadales*, *Alteromonadales*, *Burkholderiales*, *Caulobacteriales*, *Neisseriales*, *Pseudomonadales*, *Rhodobacteriales*). Some taxa show a >1.5-fold increase in hybridization intensity in the "PAH+soil" treatment (see orange and yellow highlighted samples in OTU abundance file, Figure 4.7).

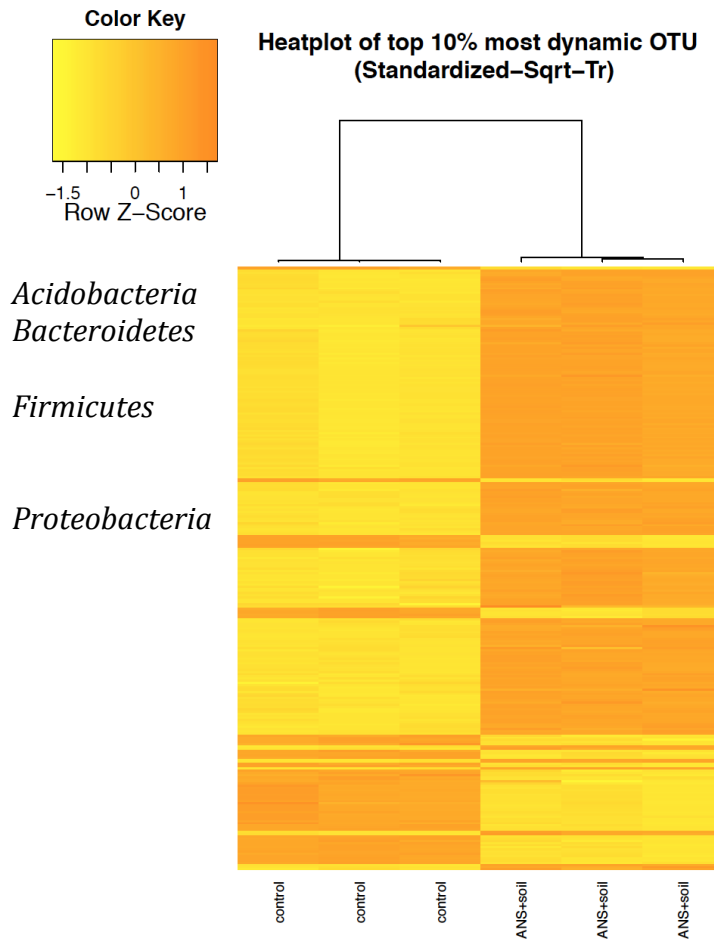
taxa_string	PAH+soil_1	PAH+soil_2	PAH+soil_3	no_PAH_1	no_PAH_2	no_PAH_3	Ave(PAH+soil)	Ave(no_PAH)	Difference
p_Bacteroidetes_o_Bacteroidales_f_Mariniflaviaceae_g_unclassified_69519**	0.18754193	0.17506307	0.18590559	0.07343262	0.0925241	0.07327775	0.182836866	0.079744824	0.10309204
p_Bacteroidetes_o_Flavobacteriales_f_Flavobacteriaceae_g_Muricauda_66546*	0.15979192	0.15832395	0.16977123	0.08435081	0.08271854	0.0795913	0.162629034	0.082220214	0.08040882
p_Bacteroidetes_o_Sphingobacteriales_f_Sphingobacteriaceae_g_Sphingobacterium_66921*	0.16919655	0.16193417	0.16369216	0.08815514	0.08872617	0.09723257	0.162530661	0.09137129	0.07115937
p_Firmicutes_o_Bacillales_f_Bacillaceae_g_Bacillus_42965*	0.16973559	0.16585339	0.17250029	0.10020443	0.10607594	0.10450146	0.169363088	0.103593943	0.06576915
p_Firmicutes_o_Bacillales_f_Bacillaceae_g_Bacillus_43066*	0.16168442	0.15341169	0.16082914	0.098498	0.1053415	0.10230987	0.158641753	0.102049789	0.06591916
p_Firmicutes_o_Bacillales_f_Bacillaceae_g_Bacillus_43506*	0.17108495	0.16446885	0.17200994	0.1020164	0.10607594	0.10632253	0.169187915	0.104804956	0.06438296
p_Firmicutes_o_Bacillales_f_Bacillaceae_g_Bacillus_43726*	0.1860545	0.18375597	0.18947925	0.12140315	0.12396304	0.12443876	0.186429905	0.123268319	0.06316159
p_Proteobacteria_o_Aeromonadales_f_Aeromonadaceae_g_Aeromonas_7119*	0.16549135	0.16226703	0.16766567	0.09976685	0.09989428	0.0991603	0.165141348	0.099607146	0.0655342
p_Proteobacteria_o_Alteromonadales_f_Shewanellaceae_g_Shewanella_6269*	0.20536726	0.20724159	0.2069144	0.11028539	0.11598191	0.11203678	0.20650775	0.112768025	0.09373973
p_Proteobacteria_o_Alteromonadales_f_Shewanellaceae_g_Shewanella_6294*	0.18676123	0.18287296	0.18869095	0.11139555	0.11762147	0.11208436	0.186108379	0.113700459	0.07240792
p_Proteobacteria_o_Alteromonadales_f_Shewanellaceae_g_Shewanella_6334*	0.23314802	0.23658083	0.23657002	0.13868732	0.1443524	0.14514794	0.235366289	0.142729222	0.09263707
p_Proteobacteria_o_Alteromonadales_f_Shewanellaceae_g_Shewanella_6607*	0.22898081	0.23236199	0.23142054	0.14248022	0.14454947	0.13943304	0.230921113	0.142154242	0.08876687
p_Proteobacteria_o_Alteromonadales_f_Shewanellaceae_g_Shewanella_6615*	0.21401433	0.21135334	0.21757728	0.12425275	0.12772627	0.13694417	0.217648316	0.128641065	0.08800725
p_Proteobacteria_o_Alteromonadales_f_Shewanellaceae_g_Shewanella_6728*	0.2205731	0.22204869	0.22008614	0.12721053	0.13009512	0.1284866	0.22092644	0.128597418	0.09230523
p_Proteobacteria_o_Alteromonadales_f_Shewanellaceae_g_Shewanella_7418*	0.23177761	0.23437678	0.23449178	0.13672144	0.14320044	0.13072884	0.233548721	0.136883571	0.09665515
p_Proteobacteria_o_Burkholderiales_f_Alcaligenaceae_g_unclassified_15153*	0.18644032	0.18562015	0.18315926	0.11790667	0.11797297	0.11751878	0.185073243	0.117799410	0.06727377
p_Proteobacteria_o_Burkholderiales_f_Alcaligenaceae_g_unclassified_15281**	0.18660336	0.18389574	0.18915119	0.08926483	0.09232811	0.09411139	0.186216764	0.09190144	0.09431532
p_Proteobacteria_o_Burkholderiales_f_Alcaligenaceae_g_unclassified_15661*	0.17748927	0.17481339	0.17556929	0.10943913	0.11212459	0.11550507	0.175957318	0.112356263	0.06360105
p_Proteobacteria_o_Burkholderiales_f_Aquabacteriaceae_g_Aquabacterium_11378*	0.16716186	0.15777122	0.16341876	0.10169424	0.10214859	0.10963124	0.162783945	0.104491357	0.05829259
p_Proteobacteria_o_Burkholderiales_f_Oxalobacteraceae_g_Massilia_15462*	0.16919849	0.16487453	0.16988826	0.10077587	0.10298124	0.1048326	0.167987092	0.102862135	0.06512386
p_Proteobacteria_o_Burkholderiales_f_Oxalobacteraceae_g_unclassified_15459*	0.22559799	0.22836911	0.2261872	0.15218691	0.14590376	0.1487046	0.226718099	0.148937253	0.07778635
p_Proteobacteria_o_Caulobacteriales_f_Caulobacteraceae_g_Brevundimonas_59158*	0.18471076	0.18098049	0.18791257	0.11554056	0.11781932	0.11415843	0.184534603	0.115839436	0.06869957
p_Proteobacteria_o_Caulobacteriales_f_Caulobacteraceae_g_Brevundimonas_59178**	0.1656071	0.16362309	0.17018046	0.10737073	0.10123244	0.09862109	0.166470216	0.102408086	0.06406213
p_Proteobacteria_o_Caulobacteriales_f_Caulobacteraceae_g_Brevundimonas_59251*	0.20183795	0.20333669	0.21310114	0.12438473	0.12444227	0.1221902	0.206091924	0.1236724	0.08241952
p_Proteobacteria_o_Caulobacteriales_f_Caulobacteraceae_g_unclassified_59760*	0.16298288	0.1625992	0.1691849	0.10660396	0.10209791	0.09756105	0.164922327	0.102087643	0.06283468
p_Proteobacteria_o_Neisseriales_f_Neisseriaceae_g_Chromobacterium_16275*	0.20480666	0.20227249	0.2091475	0.12908828	0.12817124	0.13255163	0.205408883	0.12993705	0.07547183
p_Proteobacteria_o_Neisseriales_f_Neisseriaceae_g_Chromobacterium_16426**	0.17934194	0.17295158	0.17466137	0.08191678	0.08796456	0.08874576	0.175651628	0.086209032	0.0894426
p_Proteobacteria_o_Neisseriales_f_Neisseriaceae_g_Chromobacterium_17368*	0.1705521	0.166132	0.16912616	0.1054693	0.10592946	0.10970418	0.168603421	0.107034311	0.06156911
p_Proteobacteria_o_Neisseriales_f_Neisseriaceae_g_Microvirgula_16829**	0.15536885	0.15351215	0.16199883	0.05234431	0.06357885	0.05346133	0.156959873	0.054641494	0.10049838
p_Proteobacteria_o_Neisseriales_f_Neisseriaceae_g_Vogesella_15949**	0.17990201	0.18098049	0.18706456	0.08254858	0.0864212	0.08593741	0.182649019	0.084969063	0.09767996
p_Proteobacteria_o_Pseudomonadales_f_Pseudomonadaceae_g_Pseudomonas_3451*	0.17853879	0.18142005	0.19199298	0.11931297	0.1137515	0.11281935	0.183983943	0.115294606	0.06868904
p_Proteobacteria_o_Rhodobacteriales_f_Rhodobacteraceae_g_Rhodobacter_62007*	0.16304165	0.16242529	0.17082155	0.10539149	0.10844044	0.10422044	0.165429499	0.106017457	0.05941204
p_Proteobacteria_o_Xanthomonadales_f_Xanthomonadaceae_g_Rhodobacter_17310***	0.14047114	0.13175149	0.14010905	0.21973859	0.21398589	0.22516556	0.137443893	0.219630013	0.08218612

**Figure 4.7:** Phylochip OTU abundance data. Courtesy of Lauren Tom, Lawrence Berkeley National Laboratory.

The taxa highlighted yellow show a >2-fold increase and the one taxon, highlighted blue, showed a >1.5-fold decrease in intensity (Figure 4.7). Interestingly, only one taxon showed a >1.5-fold decrease in abundance: Phylum *Proteobacteria* (Order: *Xanthomonadales*, Family: *Xanthomonadaceae*).

A heatmap, shown in **Figure 4.8** is another way to represent the same OTU abundance information provided in **Figure 4.7**. In this figure, orange means increased abundance and yellow means decreased abundance. It is a good way to

visualize changes over whole groups of taxa between our vermitea samples, Vermitea 1 “control” (no PAH) in comparison to Vermitea 2 (PAH).



**Figure 4.8:** Heatplot of the top 10% most dynamic OTU. Courtesy of Lauren Tom, Lawrence Berkeley National Laboratory.

Many of the taxa with increased relative abundances in the crude oil enhanced tea were members of hydrocarbon-degrading families. Members of these families have been shown to degrade alkanes [87] and polynuclear aromatic hydrocarbons [13, 88-91]. Interestingly, several hydrocarbon-degraders detected in the vermitea, such as *Aeromonadaceae*, *Bacillaceae*, *Pseudomonadales*, and *Shewanellaceae*, are also known to produce biosurfactants that can enhance oil degradation through gene transfer and oil emulsification<sup>19</sup> [92-94]. This experiment shows the potential for increasing the presence of biosurfactants and hydrocarbonoclastic microbial species through “priming” the vermitea with oil. This is important, since as a remediation tool, promoting the biosurfactant-producing hydrocarbon-degrading bacteria will

<sup>19</sup> Emulsification is a process in which a mixture of small droplets of oil and water is formed.

help to solubilize the hydrophobic compounds adsorbed (bound) to soil particles [72].

#### **4.4.2 Biosurfactant Analysis**

The purpose of this project was to measure the potential of surfactants present in vermitea for the remediation of hydrocarbon-impacted soils [95, 96]. The effectiveness of the application of vermitea on weathered crude oil soils may be dependent on the presence and quantity of biosurfactants [97]. The vermitea liquid extract was found to contain biosurfactant producing hydrocarbonoclastic bacteria, allowing for the increased solubility of hydrophobic compounds adsorbed to soil [97-100]. Since the addition of biosurfactants increases the surface area of the oil, this in turn increases the bioavailability of the hydrocarbon compounds, allowing the bacteria populations to more readily use the hydrocarbons found in the soil as a carbon food source [101].

##### **4.4.2.1 Halo Test**

#### **Objective**

The most commonly used methods for analyzing biosurfactant production are drop collapse, emulsification, and tensiometric evaluation [102]. Although the drop collapse test is typically the method of choice, it is not the best method to use for high-throughput screening [103]. Instead, an atomized oil assay (halo test) was performed to identify any biosurfactant producing microbial colonies [104].

#### **Methods**

Adrien Burch (UCB) [104] developed a method for plating microbes on a growth medium, then using oil to test for individual bio surfactant-producing microbes. Vermitea was plated on agar plates and microbial communities were grown for two days, then mineral oil was applied with an airbrush as a semiquantitative test for biosurfactants [104].

#### **Materials and Protocol**

Materials:

- $\text{KH}_2\text{PO}_4$  phosphate buffer
- agar plates

- mineral oil with pressurized tank
- sample solution (vermitea)

Protocol:

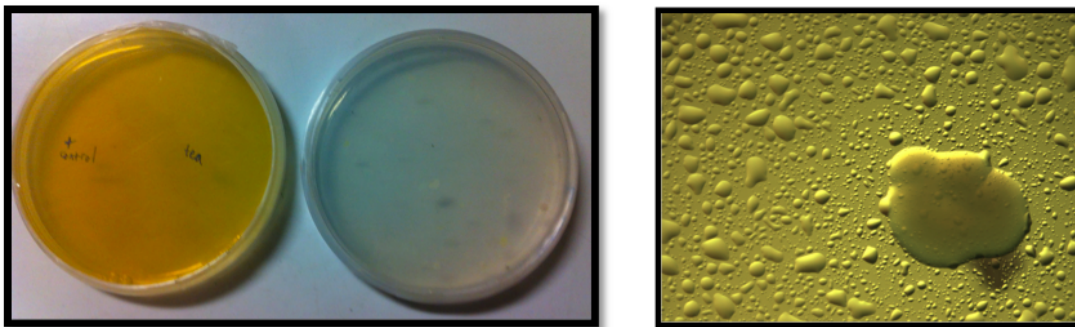
1. Plate vermitea on an agar plate.
2. Dilute the vermitea (20  $\mu$ l) with 10mM  $\text{KH}_2\text{PO}_4$  phosphate buffer (980  $\mu$ l) and plate (first dilution). This is repeated for two more dilutions (x2, and x3) and allowed to sit for two days while the microbial colonies grow.
3. Plate individual colonies on an agar plate to isolate the colonies (Figure 4.9).
4. Under a fume hood, spray mineral oil using an airbrush connected to a pressure tank. Colonies with biosurfactant producing capabilities should immediately develop “halos” surrounding the colonies.



**Figure 4.9:** Bacterial plating of vermitea showing microbial colonies.

## Results

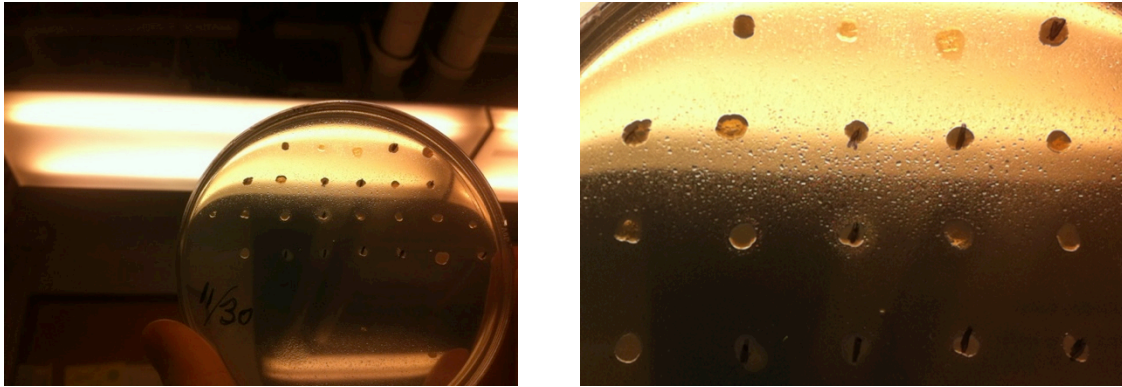
Positive results were determined by the presence of a halo, as seen in Figure 4.10, creating a reduction in the size of oil droplets near biosurfactant producing microbial colonies. The halo effect confirms the presence of biosurfactants in specific colony isolates [104].



**Figure 4.10:** Biosurfactant plating detection test showing halo formation (smaller droplets) around the microbial colony, indicating a positive result for the presence of surfactants.

The high-throughput Halo test produced no results for the control. Vermitea 2 showed at least 33 % (11 out of 30) of the isolate colonies from the treatment were positive for biosurfactant production (Figure 4.11) [104]. The initial test results demonstrated that vermitea brewed from earthworm castings with PAH (ANS)

promotes the production of biosurfactants. The next step is to determine if the presence of PAHs also promotes the growth of hydrocarbonoclastic bacteria<sup>20</sup>. A microarray analysis will compare the relative abundance of bacterial groups in the tea with and without oil and the relative abundance of taxa known to produce surfactant compounds and break down hydrocarbons.



**Figure 4.11:** Halo test for PAH-enhanced vermitea showing positive results for biosurfactants in 11 out of 30 colonies. A halo can be seen around the colonies which produced biosurfactants.

#### 4.4.2.2 Oil Spreading Test

##### Objectives

Additional surfactant qualitative and quantitative analysis was conducted to inform the best practices for enhancing the vermitea. The oil spreading test is used to detect biosurfactant production in diverse microorganisms, such as those found in vermitea [102]. The diameter of the spreading, or clear zone, is linearly related to biosurfactant concentrations. This test provides a good evaluation of biosurfactant production in multiple vermitea treatments.

##### Methods

In oil spreading, 10  $\mu$ l of crude oil is poured on 40 ml distilled water in a petri dish. Ten  $\mu$ l of the vermitea is applied to the center of the oil membrane and the area of displacement is then measured [105]. This circular area of displaced oil correlates to the activity of surfactants, which in term correlates with the concentration of surfactants in the sample solution [105]. The area (or diameter) can be related to the concentration of surfactant present with a standard curve of surfactin, a commercially available surfactant [102].

<sup>20</sup> Hydrocarbonoclastic - bacterium capable of degrading major components of oil.

## Materials and Protocol

### Materials:

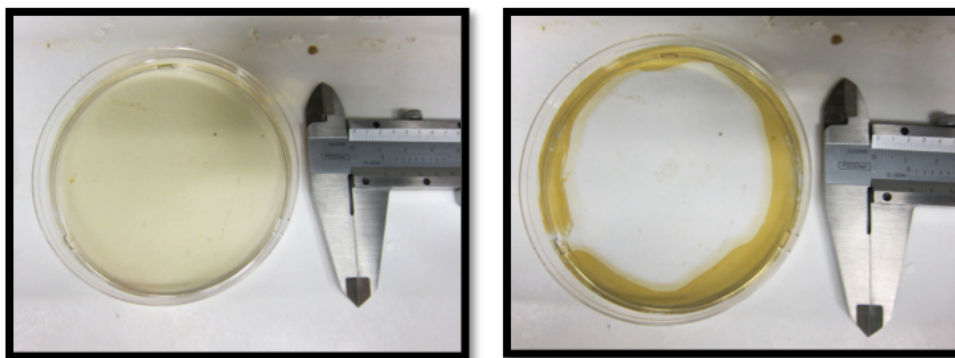
- Alaska North Slope (ANS) crude oil (10  $\mu$ l per petri dish), provided by Chevron Corporation, Richmond, California
- distilled water (40 ml per petri dish)
- petri dish (150 mm in diameter)
- sample solution (10  $\mu$ l vermitea)
- Du Nouy ring tensiometer

### Protocol:

1. 10  $\mu$ l of crude oil spread on 40 ml distilled water in a petri dish
2. 10  $\mu$ l of vermitea placed onto the center of the oil spread
3. Circular area of displaced oil is measured and correlates to the activity and the concentration of the surfactants in the vermitea [104]. Repeated in triplicate.
4. Control test with water on 10  $\mu$ l of crude oil spread on 40 ml of deionized (DI) water.

## Results

The oil-spreading test demonstrated the presence of surfactant qualities in the vermitea. The two images below show a petri dish with ANS crude oil and DI water before and after adding vermitea (Figure 4.12). The oil-spreading test produced some mixed results when trying to repeat the results from the first experiment. Replicate batches of vermitea did not always produce a positive result for the presence of biosurfactants, so future research should include a more microbial specific approach which allows the isolation and analysis of microbes.



**Figure 4.12:** Oil spreading test showing positive results for surfactants in vermitea.

### 4.4.2.3 Soil Washing Analysis

#### Objectives

The effect of vermitea on hydrocarbon concentrations in weathered crude oil soil samples by Chevron were examined by analyzing total petroleum hydrocarbons in leachate after a soil “wash” [106]. If the hydrocarbons successfully leach, the vermitea can be applied to contaminated soils with variable pretreatments, such as tilling and the addition of organic material (compost and vermicompost). The results of soil washing research will demonstrate the efficacy of vermitea as a treatment hydrocarbons bound to soil particles.

#### Methods

For the soil washing experiment, different sample types of weathered crude oil contaminated soils (artificial<sup>21</sup> and Texas field site<sup>22</sup>) were washed with the two brewed forms of vermitea in a total of three treatment groups—Vermitea 1, Vermitea 2 and Water (control). The soils were leached at 35 °C to verify that the leaching mechanism does not change over time.

Vermitea was tested for its emulsification properties on laboratory soil<sup>23</sup> which was artificially contaminated with 2 % San Joaquín Valley (SVJ) crude oil, by weight. A water bath shaker was used as the “wash” mechanism. For different vermiteas, variable amounts (2, 4 ml), shaking speeds (50, 100, 200 rpm), and times (10, 20, 30 minutes) were used. For each set of three samples, 1 gram of soil was first added to a centrifuge tube and then vermitea was pipetted into the tube. After shaking the samples for the assigned time, the soil was allowed to settle for 24-hours.

After the settling time, the vermitea was decanted and remaining oil left in the soil was extracted with hexane. 2 mL of hexane were added to each tube, shaken at 200 rpm for 5 minutes, and then pipetted out into a collection tube [106]. This process was repeated five times for each sample. The absorbances of the hexane samples were then measured on a Shimadzu Pharma-Spec UV-1700 UV-Vis Spectrophotometer at 410 nm [106].

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<sup>21</sup> Contaminated with SVJ crude oil diluted in dichloromethane (DCM), 15% of the weight of SVJ, to a final concentration of 2% SVJ crude. Soil was homogenized in a drum mixer for 24 hours, then placed in a chemical hood for 48 hours to vent and finally placed in an oven (35 °C) for 24 hours.

<sup>22</sup> Contaminated soil sample from Chevron Corporation field site in Port Arthur, Texas

<sup>23</sup> Artificial soil consists of 70% agricultural sand, 20% kaolin clay, and 10% coconut coir (by volume).



## Materials and Protocol

### Materials:

- Temperature regulated water bath shaker (set at 35 °C)
- Contaminated soil samples (1 g)
- Vermitea

### Protocol:

1. Shake test tubes with variable speeds and times.
2. After washing: contents of the test tube are allowed to settle for 24 hrs.

### Oil removal and measurement

### Materials:

- 10 mL hexane per sample
- water bath shaker

### Protocol:

1. Add 2 mL hexane to the rinsed soil and shake laterally for 5 min at 200 rpm.
2. Pipette out extract.
3. Repeat 4 times.
4. Collect all of the extract into one volumetric flask (10 ml solvent).
5. Measure absorbance of the centrifuged extract at a wavelength of 410 nm using a UV-Vis spectrophotometer<sup>24</sup>.
6. Prepare a calibration curve using known concentrations of hexane and SJV crude oil to determine the concentrations of the extracts from the absorbance values.

## Results

The initial concentration of oil was 2 % SJV by weight. From the soil washing done with castings from the Vermitea 1 (artificial oil contaminated soil) and Vermitea 2 (contaminated field site soil) bins, the overall average sample concentration of oil was reduced to 0.25 % (Vermitea 1) and 0.16.5 % (Vermitea 2) respectively (Table 4.3).

**Table 4.3:** Soil washing vermitea treatments.

Vol	Vermitea #1 (oil)	Vermitea #2 (soil)	Water (control)
2 ml	0.28 % oil left	0.19 % oil left	0.24 % oil left
4 ml	0.22 % oil left	0.14 % oil left	0.22 % oil left

<sup>24</sup> Pharma-Spec UV-1700, Shimadzu Corporation, Kyoto, Japan.

In the Vermitea 1 samples, shaking time or intensity did not affect the washing result. It is also notable for the soil Vermitea 2 samples there were downward trends in concentration related to both shaking time and intensity. Unwashed samples of soil were tested and were found to contain an average of 1.7 % oil using the hexane solvent extraction method [106].

Vermitea 2 removed a statistically significant ( $p < 0.001$ ) amount of oil in comparison to the water and Vermitea 1. However, this experiment involved only a very small time scale, and Vermitea 1 could need more time for the microbes to degrade the oil in the soil.

## 4.5 Discussion

Current commercial remediation methods, such as landfarming, require the excavation and placement of contaminated soils [18]. Landfarming has been proven effective in reducing concentrations of PAHs, but moving large amounts of soil is not always economically viable, costing more than \$100 per cubic yard [107]. The present is designed to investigate the potential for an in situ remediation treatment by researching the hydrocarbon degradation capacities of earthworms and their byproducts.

The results of the research are summarized below, including a positive result for the presence of biosurfactants in the vermitea brewed in the presence of PAHs, successful extraction of gDNA from vermitea samples for identifying specific microbial taxa, and an initial microarray analysis of two vermitea treatments. The initial microbial analysis of vermitea was followed by a vermitea leachate treatment of crude oil-impacted soil.

*Analysis and optimize hydrocarbon degraders and biosurfactant production in vermitea:*

1. Tea brewed with hydrocarbons contains a greater abundance of known hydrocarbon-degrading and surfactant producing microbial taxa
2. Microarray identified known hydrocarbon-degrading microbial taxa
3. Optimized vermitea brewing conditions for promotion of hydrocarbon-degrading taxa
4. Results were positive for the presence of surfactants capable of emulsifying crude oil and for a shift in the microbial community

Remediation was evaluated through physical experiments of contaminated soil, such as soil “washing”, and microbial community assessments of different vermitea treatments. The results were positive for the detection of biosurfactants in vermitea brewed in the presence of PAHs. Surfactants have been shown to promote biodegradation of hydrocarbons in soil [78, 95, 97]. Experiments using biosurfactants in large scale bioremediation of soil contaminated with PAHs and

heavy oil have shown significant (or complete) removal of hydrocarbons after only 22 days of bioremediation [97].

The Halo Test demonstrated more surfactant producing microbial colonies formed in oil-enhanced vermitea, but the test did not indicate which microbial taxa were involved. However, the Phylochip results demonstrated that vermitea brewed in the presence of hydrocarbons increased abundance of many known hydrocarbon degrading and biosurfactant producing microbes (*Aeromonadaceae*, *Bacillaceae*, *Pseudomonadales*, and *Shewanellaceae*) [95].

Distinct differences were observed in the microbial community composition based on initial PhyloChip microarray analysis of the vermitea samples, such as changes in the population numbers and overall morphospecies structure, or variations in phenotypes within the same species, of the microbial communities. For example, the results showed an increase in known hydrocarbonoclastic microbial taxa, such as members of the phylum *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* [99]. Members of these phyla have been shown to degrade alkanes and PAHs in previous research studies [108]. When oil was added to vermitea, these species are increasing in abundance most likely in response to the presence of PAHs.

Gulf of México water collected during the Deepwater Horizon oil spill indicated a shift in population size and diversity of native microbial communities in response to the presence of crude oil [109]. It is known that the biodegradation of hydrocarbons by microbes is one of the primary mechanisms by which petroleum and other hydrocarbons pollutants are transformed in nature [55, 110]. I hypothesize a similar response in vermitea that hydrocarbon-utilizing microorganisms would populate in the presence of oil. Additional research will help to determine better the value of PAH stimulated vermitea as a remediation tool.

## **Conclusions and Future Research**

Based on this initial research, it appears that vermitea has the potential to be further optimized by exposing earthworms and microbial communities to low levels of PAHs prior to their use for remediation. Enhancements could include manipulations that increase the presence of biosurfactants and hydrocarbonoclastic microbial species, such as “priming” the vermitea with oil. As a remediation tool, promoting the biosurfactant-producing hydrocarbon-degrading bacteria may help increase solubilization of the hydrophobic compounds adsorbed to the soil [72]. This is especially important when treating environmentally persistent, recalcitrant high-molecular-weight PAHs, allowing bacterial populations to break down and use the hydrocarbons as a carbon source [16, 21, 24, 111]. Additional microbial analysis, of additional vermitea samples and soil treatments, will help to understand the relationship between shifts in microbial communities and earthworm exposure to

crude oil and how these treatments contribute to a viable vermiremediation technology.

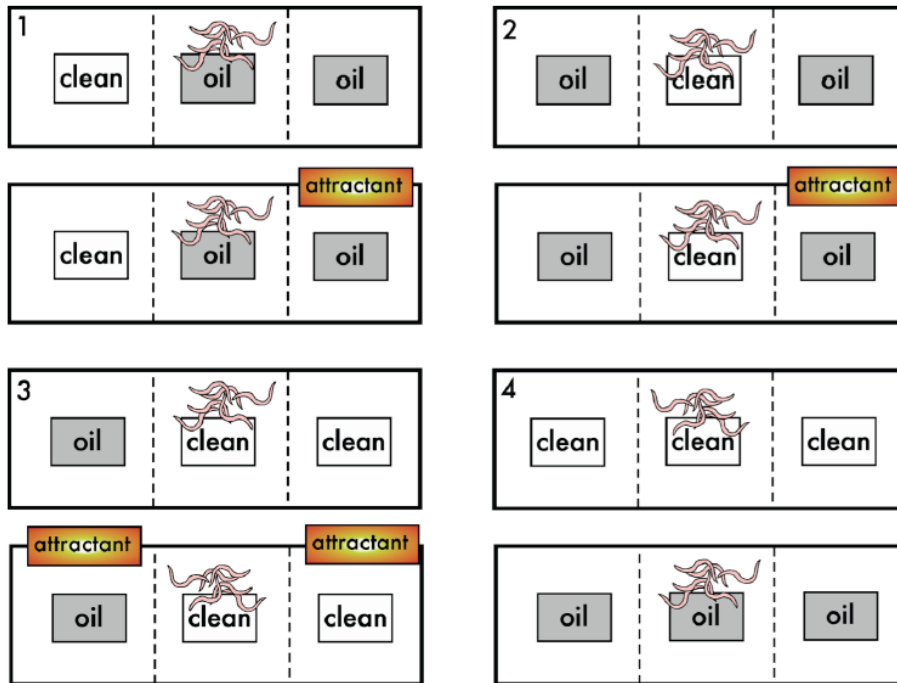
Evolutionary theory suggests that certain environmental pressures, such as toxic chemical exposure, may select for individual organisms within a community which are best adapted to survive [112]. Future research would include the evaluation of *E. fetida* earthworms that have survived high crude oil exposure for improved PAH tolerance and degradation capacity. Previous research has shown that a few earthworms (~10 %) survived exposure to 10 % crude oil by weight in soil [113, 114]. My research results demonstrated tolerance by *E. fetida* up to 40 mg/kg fluorene, 200 mg/kg phenanthrene, and 5 % by weight San Joaquín Valley (SJV) crude oil. Worms surviving 2 %-10 % crude oil may be more tolerant to hydrocarbon exposure because of genetic characteristics contributing to hardiness and microbial partners in the gut [48].

It may be possible to select earthworms that are more tolerant to oil exposure than the average population, test their ability to produce equally hardy offspring and improve degradation of crude oil. With this process one aims to select for a population of earthworms that is more suitable for degradation of crude oil in soil than the average composting worm. *E. fetida* that survive higher levels of crude oil exposure can be maintained separately and kept in cultivation with 1-2.5 % crude oil exposure. The level of crude oil can be adjusted based on earthworm performance to reach the maximum possible levels that the worms will continue to grow and reproduce. *E. fetida* that have been cultivated in the presence of crude oil may enhance the efficacy of the vermiremediation technology.

Ecotoxicological assessments of earthworms can be tested using migration and avoidance behavior experiments [115]. An avoidance behavior test evaluates whether or not earthworms will prefer to leave a contaminated site, or would remain there. There is also a potential for “lures” to be used in oil-contaminated soils when earthworms are given options between non-contaminated and contaminated soils [116]. *E. fetida* have chemoreceptors in the prostomium (lobe above the mouth) and sensory tubercles on their body surface, which helps them to detect chemicals in the soil [117]. Adding lures for earthworms to a contaminated soil may give them incentive to stay in soil, thus promoting remediation. This may be a critical aspect of developing a successful field treatment using earthworms. One demonstrated attractant for *E. fetida* is a commonly occurring soil fungus, *Geotrichum candidum* [118]. *G. candidum* is commonly found in leaf litter, indicating it may be feasible to use this fungus, or its extracts, as an attractant for worms. The potential soil preference of *E. fetida* can be evaluated by observing their movement when provided oil-contaminated and clean soils.

An artificial soil mix contaminated with crude oil can be placed into a segmented box, varied configurations, to test the behavior of the worms when given specified choices (Figure 4.13). Avoidance behavior tests commonly use a plastic divider for separation of the soils during bin setup, which is then removed to allow migration

[115, 116, 119]. However, soils could also be separated by a screen through which worms may migrate, but that keeps soil treatments separated.



**Figure 4.13:** Potential configurations of test bins for avoidance behavior and attractant tests. Dashed line indicates screen divider or removable split between soils.

There are many other important aspects of the vermiremediation process that require better characterization prior to successful adoption as a soil treatment technology. Systematic comparisons of PAH degradation between vermi-compost and other remediation technologies, such as landfarming is required [20]. For scaling the technology across geographic borders, research should be done to understand the influences of worm feedstock quality on the outcomes of vermiremediation in different countries, regions, or continents. Feedstock composition influences activity of bacteria soil, so different sources of organic matter may determine the outcomes of vermiremediation of PAHs [62, 63]. Reports show that different organic matter sources influence the vermiremediation of PAHs [5, 6]. The total earthworm biomass needed for effective vermiremediation of a field site is important to determine prior to implementation of the technology [60, 63]. The necessary earthworm biomass will be related to the concentration of PAHs and soil conditions [63].

Bioremediation is one of the most promising processes for cleaning up oil spills in soil [120, 121]. In areas where natural attenuation of PAH-contaminated soils is inhibited because of low bioavailability of substrate, nitrogen, or other nutrients,

required hydrocarbon-degrading microbes, or because of heavy, recalcitrant hydrocarbons [8, 11], landfarming technology is commonly done [17]. Landfarming helps to stimulate the degradation capabilities of natural microbial communities in soil by the addition of compost amendments and physical mixing (tilling). The success of landfarming, however, is limited by many factors, such as microbial population density, soil pH, nutrient concentrations, and the chemical structure and biodegradability of the contaminants [10, 19]. The results of this preliminary work with earthworms and their byproducts demonstrates the potential for vermiremediation technologies to move beyond the limitations of landfarming and increase biodegradation rates in petroleum-impacted soils at an application scale.

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## Chapter 5

### Conclusions

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#### 5.1 Oil Spills

There is a high risk for environmental damage due to oil and gas industry activities. The industry operates in extreme climate conditions and remote locations. The occurrence rates for oil spills are great due to the large volumes of oil being processed worldwide, coupled with the causes for a spill: equipment failure, adverse weather conditions, and human error.

A principal goal of this dissertation is to provide an overview of the Deepwater Horizon oil spill response—conventional remediation technologies, alternative technologies evaluated during the spill, and the cleanup effort. As was the case of the Deepwater Horizon event, much of the oil is not recovered and lost to the environment. Therefore, the dissertation also includes an investigation of microbe-facilitated remediation of oil-contaminated soil and the development of marsh shoreline restoration innovations.

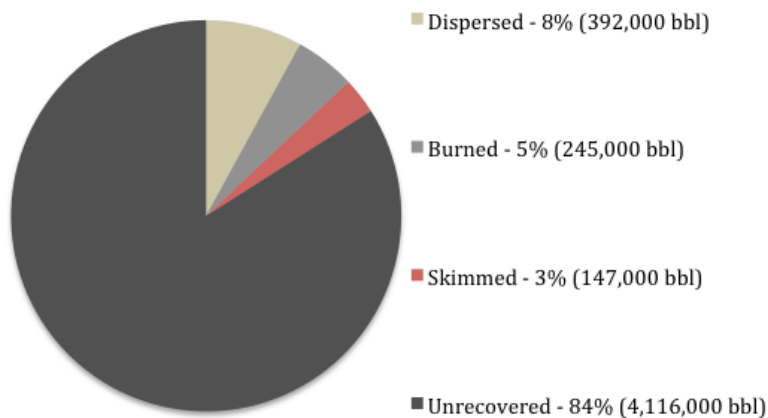
#### 5.2 Oil Spill Response

The 2010 Deepwater Horizon oil spill set in motion an ongoing environmental disturbance in the Gulf of México that is unprecedented in scope. Response efforts made use of chemical dispersants, sand berms, booms, and vessels of opportunity in attempts to recover oil or render it less harmful. The total discharge of oil from the spill is estimated to be 4.9 million barrels (1, 2). However, British Petroleum (BP) installed a 10.16 cm insertion tube into the leaking 50.8 cm riser pipe which effectively “captured” 850,000 barrels of oil before it was released into the Gulf waters (3). Therefore, technically, 4.05 million barrels were released (into the water) and a fine was assessed accordingly (4).

Oil spills related to production and transportation activities are a common occurrence. It is our responsibility to care for the environment by developing tools for effectively responding to a spill. Oil spill response strategies are based on the physical property of oil being buoyant in water. Crude oil remains on the surface of water until it undergoes chemical and physical changes (5, 6). The Deepwater Horizon response employed skimmers for oil removal, chemical dispersants to move oil into the water column, and in-situ burning which releases oil into the atmosphere (7).

The case study of the Deepwater Horizon oil spill examines reasons for our inability to recover oil from the environment. The limits of conventional oil skimming technology led to the greatest application of chemical dispersants (6.8 million liters) and in-situ burns (410 burns) in the history of spill response (8). Despite the massive spill effort, only 16 % of the 4.1 million barrels of oil was affected by the response (Figure 5.1).

For hazardous material spills in the United States, other than oil, the response policy does not include any reference to a protocol where dispersion of the toxic substance as an acceptable strategy. The objective of incident management is always to seek a net environmental benefit, so any substance spilled in the environment must be contained and removed (9). Section 300.415(b) (3) of the National Oil and Hazardous Substances Pollution Contingency Plan states, “If the lead agency determines that a removal action is appropriate, actions shall, as appropriate, begin as soon as possible to abate, prevent, minimize, stabilize, mitigate, or eliminate the threat to public health or welfare of the United States or the environment” (10).



**Figure 5.1:** The Deepwater Horizon Oil Budget<sup>1</sup>

The Deepwater Horizon case study helps to establish the gap between acceptable mitigation strategies for oil spills (disperse and burn) and other toxic material spills (contain and remove). The research concludes there is a need for additional investment in oil spill preparedness, development of environmentally sound technologies (ESTs)<sup>2</sup>, and improved restoration strategies (11-13). The Deepwater Horizon research serves as a primer for understanding environmental tradeoffs associated with oil spill response decisions. The proceeding sections of the

<sup>1</sup> Lehr, B., S. Bristol, and A. Possolo, Oil Budget Calculator: Deepwater Horizon, 2010, The Federal Interagency Solutions Group, Oil Budget Calculator Science and Engineering Team: <http://www.restorethegulf.gov>.

<sup>2</sup> Technologies that have the potential for significantly improved environmental performance relative to other technologies.



dissertation provide the basis for continuing this discussion on the fate and consequences of oil in the environment.

### **5.3 Remediation and Restoration**

Contamination by oil spills of coastal ecosystems, especially sensitive shoreline ecosystems, such as marsh sediments, has been common in countries with a productive oil industry (14). The development of models and remediation processes for application to marsh sediments affected by petroleum hydrocarbons and heavy metals has become an important technological requirement in countries with coastal regions and oil production activities (15). A remediation tool to be included in contingency plans for oil spills is needed, especially regarding ecosystems with ecological and economic importance such as marshes, ranked as one of the most sensitive habitats in the world in the NOAA Environmental Sensitivity Index<sup>3</sup>.

Marshes represent some of the most productive ecosystems on earth, providing habitat to migratory birds and diverse waterfowl, as well as fish, shellfish, and numerous other species (16, 17). They also provide important ecosystem services to humans beyond the more than \$100 billion dollar tourist industry. For instance, coastal marshes function to ameliorate flooding and buffer the coast from storms, providing an estimated 2.8 billion dollars of protection per year to Louisiana and benefiting 14 million Gulf Coast residents vulnerable to hurricanes (18).

***Everyone to whom much was given, of him much will be required***  
***- Luke 12:48***

Shoreline interventions included construction of sand berms and freshwater diversion to impede the onslaught of oil. It is not clear the value of these strategies because very few data were collected to substantiate their use (19). However, vegetative transplantation has been verified as an effective means to restore oil-contaminated wetlands and accelerate oil degradation in soil (20, 21). My collaborative research effort in Louisiana, described in Chapter 3, demonstrated alternative methods of oiled marsh restoration. An oiled marsh in Barataria Bay, Louisiana, one of the most heavily oil-impacted coastal systems, was selected for a comprehensive restoration research project (22-24). The project emphasized the use of locally-adapted, genetically-diverse smooth cordgrass, *Spartina alterniflora*, in place of the State mandated cordgrass cultivar, *Vermillion*, and the addition of a sugar cane industry waste byproduct, bagasse, as a growth medium (22, 25, 26). The marsh study will further confirm supporting killed and eroded vegetation with bagasse and native *Spartina alterniflora* can promote marsh regrowth and limit the ecological consequences of oil pollution (20, 21, 25). The treatment study and

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<sup>3</sup> <http://response.restoration.noaa.gov/maps-and-spatial-data/shoreline-rankings.html>

follow-on restoration studies in Bay Jimmy represent important steps towards achieving similar outcomes for Gulf coast marshes.

***“God made oil float so it could be recovered”***  
***– Kevin Costner***

The Alternative Response Technology Evaluation System (ARTES) program was employed during the Gulf of México oil spill event (27). Normally reserved for evaluation of technologies other than mechanical cleanup methods, ARTES was used to test and adopt new tools, including oil recovery technology (28). Conventional weir skimmers' recovery rate is dependent on the type of oil, sea state, and the ability to contain enough oil for the period of time necessary for recovery (29, 30). More than 2,000 skimmers were deployed, capturing only 3 % of the oil (17.5 million liters), making oil-water separation technology, such as the Costner centrifuge, a practicable part of the recovery effort (7). The Deepwater Horizon case study demonstrated that alternative technologies played a key role in the helping to mitigate damage to the Gulf waters and coastal systems.

## **5.4 Bioremediation of Crude Oil in Soil**

Removing oil contamination from soil is a difficult and requires very different techniques from removing oil from water. Unlike water, oil binds to soil particles, limiting surface area, bioavailability, and thus the potential for natural attenuation (31, 32). The results of our soil remediation research were positive, demonstrating that vermiremediation should be considered as a viable as part of a larger, remediation strategy. We discovered biosurfactants in vermitea brewed in the presence of polynuclear aromatic hydrocarbons (PAHs). Surfactants have been shown to promote biodegradation of hydrocarbons in soil (33-37). Experiments using surfactants in large scale bioremediation of soil contaminated with PAH and heavy oil have shown significant (or complete) removal of PAHs after only 22 days of bioremediation (35).

The research also demonstrated an increase in surfactant producing colonies forming in oil-enhanced vermitea. The microarray analysis showed in these same teas many of the bacteria which increased in abundance also are known to form biofilms and may produce biosurfactants (*Aeromonadaceae*, *Bacillaceae*, *Pseudomonadales*, and *Shewanellaceae*) (37). It can be concluded that oil-enhanced tea stimulates an increase in the abundance of known surfactant producing bacteria. Earthworms ingest soil microbial populations that change with transit through the gut (38, 39). Some are stimulated while others are eliminated prior to exiting the gut as casts, directly impacting the soil microbial community (39). This microbial community likely changes composition when exposed to crude oil, but this has not

been well-documented, and only one study examining the total microbial phospholipid fatty acid profiles was able to detect a clear shift (40).

Vermiremediation uses natural earthworm processes—aeration, soil mixing, increased microbial activity, increased bioavailability—compared to conventional approaches which may increase harmful side-effects on the surrounding environment (41, 42). Survival of some earthworms at higher concentrations of PAHs might be explained by successful adaptation of the microbial communities in the guts of these individuals (43). Determining the change in earthworm microbial communities in response to feeding on oil-contaminated soils may inform environmentally sound remediation strategies. Identifying key taxa of the PAH-degrading community may enable future design of earthworm feed that will promote known PAH-degraders.

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