Vermitea Remediation of Hydrocarbon Contaminated Soil

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ABSTRACT

Due to the global need for oil production and distribution, surrounding ecosystems have been negatively affected by oil spill externalities in individual health and community diversity. Conventional land remediation techniques run the risk of leaving chemical residues, and interacting with metals in the soil. The objective of this study was to test worm compost tea, also known as vermitea, as a bioremediation method to replace current techniques used on oil contaminated soils. To test the conditions that contributed to the efficacy of the teas, I examined different teas that looked into the mode and length of pollutant exposure. I examined oil emulsification activity, presence of biosurfactant-producing bacteria colonies, microbial diversity and abundance, and applicability of the teas to artificially contaminated soils. Overall, I found that the long-term direct oil tea had a 7.42% significant increase in biosurfactant producing microbes in comparison to the control tea. However, the long-term crude soil vermitea was found to be the best type of pollutant degrading tea in terms of emulsifying activity and general applicability towards reducing oil concentrations in the soil. These results will help broaden the scientific understanding towards stimulated microbial degradation of pollution, and broaden the approaches that can be taken in restoring polluted ecosystems.

KEYWORDS

bioremediation, microbial populations, biosurfactant, emulsification, soil pollution

INTRODUCTION

The global demand for crude petroleum has contributed to detrimental effects on surrounding ecosystems. Petroleum is predominantly made up of hydrocarbons, organic molecules that can be lethal in ecological contexts (Tang 2011). Large tanker oil spills and other accidental discharges of petroleum have negatively impacted sea life and polluted land near the spills, creating crude oil contaminated soils (Shaw 1992). After the 1989 Exxon Valdez oil spill, the persistence of hydrocarbons along Prince William Sound coast caused the destruction of surrounding habitat. However, this event also marked the introduction of bioremediation as a mainstream treatment of polluted bodies of water and soil sites (Hoff 1993).

Bioremediation is the use of biological organisms to facilitate the degradation of environmental pollutants (Iwamoto 2001), and can be enhanced through modifications to site conditions such as temperature, pH and nutrient availability (Hoff 1993). The bioremediation of petroleum hydrocarbons is facilitated by hydrocarbonoclastic bacteria, microbes capable of breaking down organic pollutants into simple carbons (Jagadevan 2004). Hydrocarbonoclastic and other strains of bacteria can also produce biosurfactants that enhance the removal of oil in the environment. Biosurfactants are surface-active molecules that emulsify hydrocarbon molecules in the polluted medium, increasing the bioavailability of the pollutant for microbial consumption (Jagadevan 2004). Different biological approaches in remediation have drawn from many disciplines, such as bioaugmentation, the addition of an assortment of microbial populations (Ayotamuno 2007); landfarming, the addition of polluted soils to a soil surface that is then tilled for aeration to promote microbial activity (Marin 2004); and the application of natural and synthetic fertilizers to increase pollutant degradation rates through microbial nutrient availability. This study attempts to apply a method from the discipline of organic farming, the use of vermitea, in order to select for microbial activity adapted to soil pollutants.

Vermitea, a liquid mixture of vermicompost, is a supplement for enhancing crop growth rate and nutrient availability (Magpantay 2010). Conventional vermitea used in farming incorporates worm castings containing microbial populations from earthworm intestines. In preparation for soil application, microbial mixtures are aerated for a period of time to increase bacterial population growth. In this experiment, worm castings, water and supplemental nutrients were aerated, or “brewed,” reflecting the method of organic farming. However, the castings used

in the study were produced by worms that were lightly exposed to petroleum hydrocarbons; exposure to oil was manufactured in order to stimulate the growth of hydrocarbon-degrading and surfactant-producing intestinal microbes (Lindstrom 1999). Although commercial surfactants have conventionally been used to augment the degradation of pollutants, they can be problematic for the ecosystems they are meant to remediate (Joshi 2010). Surfactants can act as disinfectants against certain beneficial bacteria (Plante et al 2008) and also have long ecological lifetimes (Joshi 2010). Because conventionally used tools for hydrocarbon remediation may have negative impacts of their own, this study aims to compare vermiteas created through different oil exposure methods as potential replacements for surfactants.

The objectives of this study are to test vermitea as a potential bioremediation implement for crude oil contaminated soils, and to determine the effect of oil contamination on microbial populations. The study will address the overall effectiveness of vermitea against oil contamination by testing the emulsification ability of the tea and applying different versions of the tea to oil polluted soils. The study will also address the effect of prior hydrocarbon exposure on the microbial diversity and biosurfactant production in the vermiteas. I hypothesize that there will be less diversity but heightened abundances of hydrocarbon-degrading microbes in the teas brewed from oil-contaminated worm castings in comparison to the control brewed from regular castings (Lindstrom 1999). I also hypothesize that there will be a higher surfactant concentration in the oil-exposed worm casting teas in comparison to the control due to expected increases in hydrocarbon degrading microbes. I hypothesize that the oil-exposed teas will facilitate higher oil degradation rates than the control, complementing the expected increase in hydrocarbon degrading microbes and biosurfactants. In addition to hydrocarbon bioavailability, I hypothesize that the oil-exposed vermitea will be effective in emulsifying and degrading hydrocarbons from the soil.

METHODS

Worm bin establishment

For uniform worm castings, I filled three 0.117 m^3 compost bins each with 2.6 kg of *Eisenia fetida* earthworms and castings, and an additional 200 adult worms to boost population

numbers, all sourced from the UC Berkeley Richmond Field Station (Richmond, CA). I then added 200 g of compost bedding from stable waste from the Riverside Equestrian Center (Petaluma, CA) and 500 mL of water to help the worms adjust to feeding on the organic matter. The three bins were differentiated by weekly inputs of feedstock across a five week period: the control bin (bin 1) was fed with uncontaminated compost bedding; the second "direct oil" bin (bin 2) was fed with compost bedding contaminated with San Joaquin Valley (SJV) crude oil from Chevron Corporation (Richmond, CA); the third "crude soil" bin (bin 3) was fed with compost bedding contaminated with weathered crude soil from an oil-polluted site in Port Arthur, Texas.

Preparation of feedstocks

I created the oil feedstock by adding 16.5 g of SJV crude oil—2% of the compost bedding dry weight—with 3.03 g of dichloromethane (DCM) into 1274.74 g of compost bedding. As SJV crude is inherently weathered, I used the oil in this experiment to reproduce contaminated field conditions. The contaminated feedstock incubated in the fume hood for 72 hours at room temperature to vent away small volatile hydrocarbon molecules. Equal parts of DCM and compost bedding were prepared for the control and crude soil bins (bins 1 and 3, respectively), and also incubated in a fume hood at room temperature for 72 hours. After the incubation period, 242.9 g of crude soil was added to the feedstock for bin 3. For the weekly feedings, approximately 210 g of feedstock were added to the appropriate bins as a thin layer atop the material already present; 1L of water was added to each of the bins after adding the feedstock. After the feeding period, I waited another five weeks for the feedstock to be processed by the worms; castings were collected by hand from the bins to create the vermiteas.

Vermitea brewing

A bench top vermitea aeration system was set up using 500 mL conical separatory funnels. A vermitea sample was started with 500 mL of deionized Milli-Q water, 600 μL each of fish hydrolyzate (Earthfort, Corvallis, Oregon) and of kelp fertilizer (Sanctuary Blend, Monterey Bay, California), and 0.1 g of humic acid (Down to Earth, Eugene, Oregon). Fish hydrolysate

and kelp fertilizer were added as nutritional inputs for the microbial populations. Subsequently, 9.76 g of wet worm castings from the appropriate bin were press-filtered into the liquid mixture and the mix was poured into a separatory funnel. The vermiteas were aerated at 2.0 psi for 24 hours at room temperature in order to boost microbial population growth and activity.

Types of vermitea investigated

In the study, I examined the effects of five different types of vermitea on oil-contaminated soils: 1) tea aerated with castings from the control bin (bin 1); 2) tea with castings from the control bin (bin 1) with additional SJV crude oil added; 3) tea from the oil contaminated bin (bin 2); 4) tea with castings from the control bin (bin 1) with additional crude soil added; and 5) tea from the soil contaminated bin (bin 3) (Table 1).

Table 1: Summary of vermitea types and additions

Type of vermitea	Bin	Additions before aeration
1) Control bin	9.76 g control castings	None
2) Control bin and oil	9.76 g control castings	0.0525 g SJV crude oil
3) Control bin and soil	9.76 g control castings	0.73 g SJV soil exposed
4) Oil bin	9.76 g oil exposed castings	None
5) Soil bin	9.76 g SJV soil exposed castings	None

Emulsification and halo tests

I treated emulsification ability of vermitea as a major contributing factor to the degradation of hydrocarbons in contaminated soils (Banat et al 1995). I employed an E24 emulsification test that allowed for the determination of the emulsifying abilities of each vermitea type (Ilori 2005). For each tea type, I ran triplicate samples. I pipetted 1 mL of each replicate into a test tube containing 1 mL of Alaskan North Slope (ANS) crude oil (Chevron Corporation, Richmond, CA) and then vortexed for two minutes. Instead of SJV crude, the less viscous ANS oil was used to increase the range of emulsification values observed from the replicates. After a 24-hour incubation at room temperature, I measured the heights of the

emulsification layer and the entire liquid layer to produce emulsified layer percentages. Emulsification fractions above 50% were considered to be positive for emulsification by the tea sample.

I also used a plating detection method to assess the number of biosurfactant producing colonies from each vermitea type. For each tea type, I ran triplicate samples. Ten μL of each vermitea sample was pipetted and spread onto a 10% trypticase soy agar petri plates. After incubating three days at room temperature, I transferred each individual colony on the plates onto new petri plates to increase individual colony visibility. Each new plate contained a positive control (wild type *Pseudomonas syringae*, known biosurfactant producer) and a negative control (syfA-/rhlA- *Pseudomonas syringae*, with no biosurfactant activity). After re-plating the colonies, I sprayed mineral oil (light paraffin oil, Fisher Scientific) onto each plate using an airbrush (type H; Paasche Airbrush Co., Chicago, IL). Any surfactants produced by bacterial colonies reduced the size of the oil droplets on the petri plate surface, forming visible halos (Burch 2010). With the halo results from the plates, I was able to gauge the relative biosurfactancy of the vermitea types as well as isolate biosurfactant colonies.

In addition to testing biosurfactant production, I also noted the absolute number of colonies and the types and abundances of the microbial strains in terms of morphology.

Soil Washing

To evaluate the applicability of the different vermiteas, I washed 2% SJV crude oil contaminated soils with each of the teas and measured oil concentrations before and after vermitea application. To prepare the soil, I sieved soil collected from the Richmond Field Station to remove large pieces of organic matter. I then added 7.19 g of SJV crude oil and 1.52 g of DCM, and rotary-mixed 419.27g soil with the oil for 24 hours. After mixing, I left the soil in a fume hood to vent for 48 hours and lightly oven weathered the soil for 24 hours at 50^{o}C (Urum et al. 2004). For each vermitea type, I separated 54, 1g soil samples into two groups, to be washed with 2 or 4 mL of tea to measure the effect of washing volumes. In each group, samples were further divided into low, medium, and high shaking speeds and shaking times in order to measure the effect of shaking magnitude and time and to determine the best physical conditions to remove the oil (Table 2).

After washing, the tubes containing the liquid and soil were allowed to settle for 24 hours, and the liquid was subsequently decanted. I determined the oil remaining in the samples with hexane solvent extraction. Ten mL of hexane was added to each rinsed soil sample and shaken for 5 minutes before transferring the hexane-oil extract to a centrifuge tube; this process was repeated three more times (Urum 2004). After centrifuging the extracts at 3000 rpm for 10 minutes, I measured the absorbance of the extracted samples with a UV-Vis spectrophotometer at a wavelength of 410 nm (Pharma-Spec UV-1700, Shimadzu Corporation, Kyoto, Japan). The maximum absorbance was observed to be at 410 nm with a standard mixture of hexanes and SJV crude oil. I prepared a calibration curve using known concentrations of hexane and SJV oil to determine the concentrations of the extracts from the absorbance values. The absorbance concentration conversion was calculated to be

Crude oil removed (%) = [0.0099*(Absorbance)-0.0005] * 100%.

Between the three shaking speeds used in the study, I found 100 rotations per minute (rpm) significantly decreased the residual oil in the soil in comparison to 50 rpm, and 200 rpm also resulted in significantly lower oil concentrations than 50 rpm (1-way ANOVA, $F(2,321) = 34.03$, $p < 0.001$) (Figure 3).

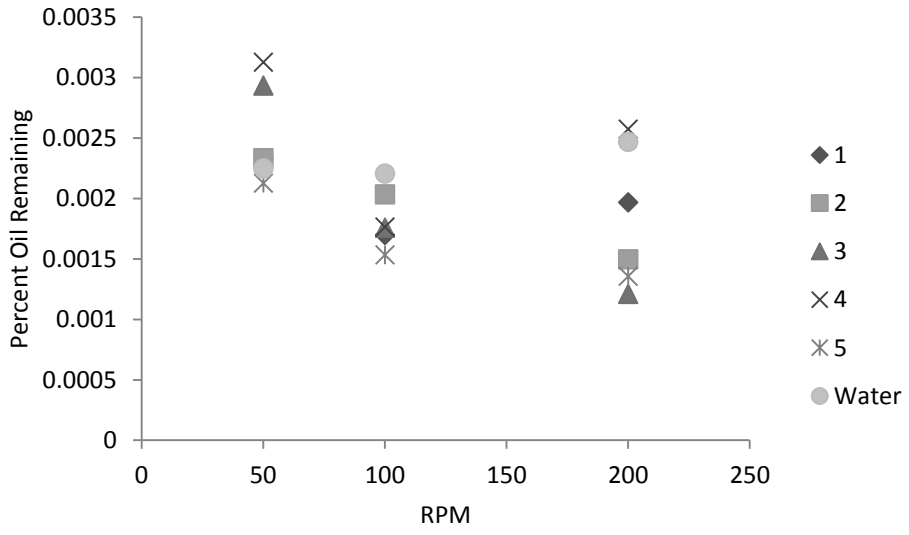

Figure 3. Relation between RPM (rotations per minute) and residual oil. As the speed of the shaker was increased, there was a corresponding increase in the removal of hydrocarbons from the soil samples.

In examining the five vermitea samples, I found agitation time to be significant between 10 and

20 minutes, and also between 10 and 30 minutes of using the shaker (1-way ANOVA, $F(2,321) = 8.104$, $p < 0.001$) (Figure 4).

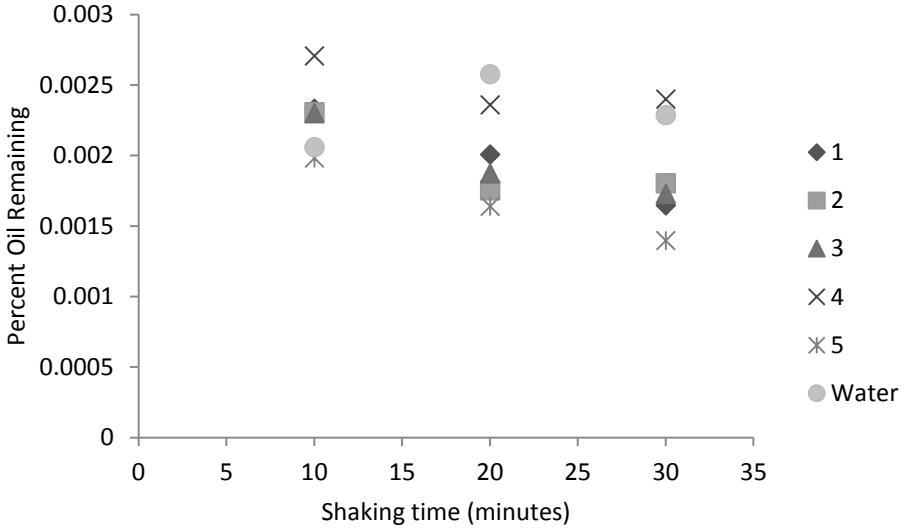

Figure 4. Relation between shaking time and residual oil. As the shaking time was prolonged, there was a corresponding increase in the percent of oil removed from the soil samples.

Statistical analyses

I conducted ANOVA and Tukey HSD post-hoc tests between the vermitea treatment groups and the control to examine potential differences in the emulsification ability of the different vermiteas. Due to non-normalities in the distribution of my data, I conducted Kruskal-Wallis one-way analyses of variance on the biosurfactant production and soil washing abilities of the vermitea types. For significant results from the Kruskal-Wallis analyses, I used Wilcoxon rank sum tests to determine which group pairs were statistically different. I also calculated Shannon-Weiner diversity indices from the colony morphospecies data in order to interpret the community composition seen in the different vermitea plate replicates. I was interested in quantifying and comparing the diversities of the vermiteas as diversity can be attributed to the general stability and health of communities (Hill 1973). With the Shannon-Weiner indices, I conducted an ANOVA to identify if there were differences between the diversities of the vermiteas.

RESULTS

Emulsification ability of vermiteas

The five vermitea types displayed oil emulsification activity at a ratio of 0.71 ± 0.08 (mean ± SD). The two crude soil exposed vermiteas (5, 3) had the highest activity with 0.76 ± 0.08 and 0.74 ± 0.09, respectively. Emulsification ability was, on average, 5.7% higher in the long-term direct oil vermitea (4) and 10.7%, in vermitea 5 with respect to the control vermitea (1) (Figure 1).

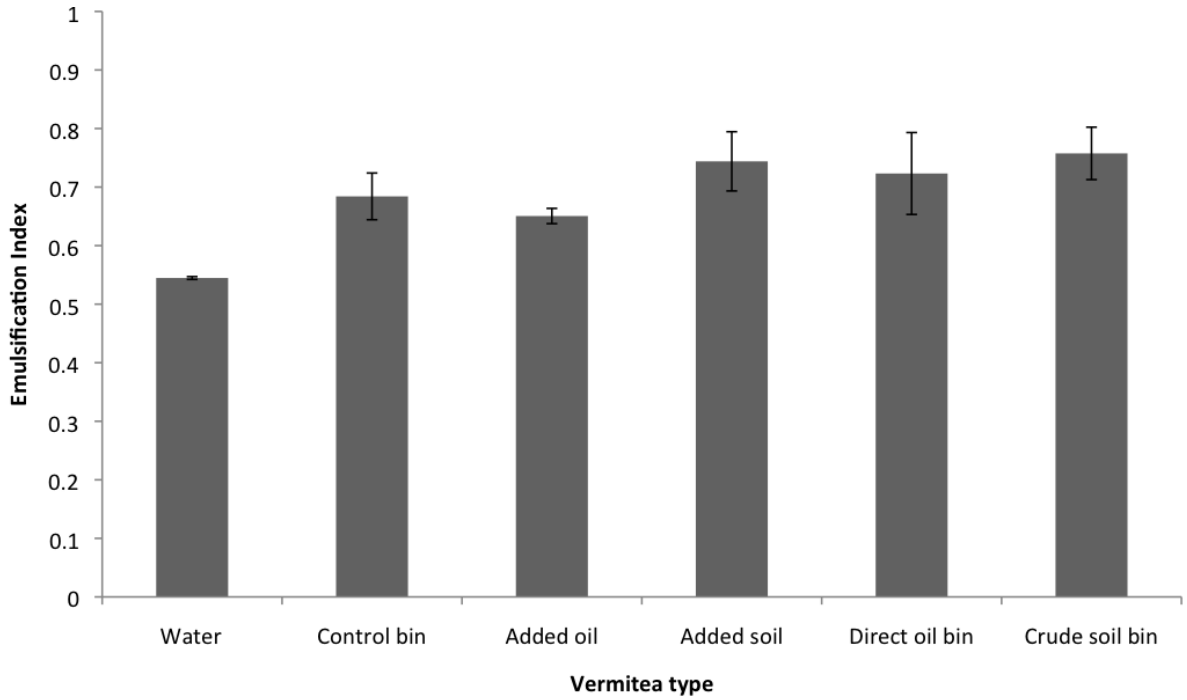

Figure 1. Comparison of emulsification (E24) abilities of the different vermiteas (castings from control (1), oil (4), soil (5) bins and control castings with added crude oil (2), and with added Texas crude soil (3) compared to the water-only negative control. The long-term vermiteas (4, 5) had significantly higher emulsification averages than their short-term counterparts (p=0.0411).

Between the five vermitea groups and the water negative control, I found a significant differences in emulsification capabilities (1-way ANOVA, $F_{5,12} = 3.32$, p=0.0411). Vermitea 5 had an average emulsification value 39.1% higher than the water baseline (Tukey HSD, p = 0.0411).

Biosurfactant colony abundance and community structure

The long-term direct oil vermitea (4) triplicates had an average of 7.4 ± 6.6% biosurfactant-producing strains, out of 26.3 ± 9.3 individual colonies. The relative abundance of biosurfactant-producing colonies in vermitea 4 was 7.9 times greater in comparison to vermitea 3, and 4.1 times greater in comparison to the long-term crude soil vermitea (5) (Table 1). Both the control vermitea (1) and the short-term direct oil vermitea (2) did not test positive for the presence of biosurfactant-producing bacteria.

Table 1. Relative abundances of biosurfactant-producing bacteria in vermiteas (mean ± SD). The majority of the pollutant pre-exposure vermiteas (3,4,5) tested positive for biosurfactant-producing colony presence. The long-term direct oil vermitea (4) had the highest significant difference in relative biosurfactant-producing colony abundance in comparison to the control vermitea (1), which did not test positive for biosurfactants (p=0.369).

Vermitea Type	% Biosurfactant-producing	Total colonies
1	0	39 ± 2
2	0	28.33 ± 13.58
3	0.83 ± 1.44	35 ± 8.66
4	7.42 ± 6.63	26.33 ± 9.29
5	1.45 ± 2.51	27.33 ± 4.51

There was a difference between the absolute abundance of biosurfactant producing bacteria (Kruskal-Wallis, $H_4 = 9.5814$, $p = 0.0481$); within the groups, vermitea 4 had a significantly higher abundance than the two vermiteas (1 and 2) that tested negative for biosurfactant colonies (Wilcoxon rank sum, p=0.369) and the short-term crude soil vermitea (Wilcoxon rank sum, p=0.0463). Overall, vermitea 1 had the highest average strain abundance, with 39 ± 2 colonies, 11.43% higher than the second highest strain abundance, which came from the short-term crude soil vermitea (3). With both short and long term exposures to oil pollution, both colony abundance and diversity decreased. For both the direct oil and crude soil long-term exposures (4, 5), diversity was diminished by 10.5% in comparison to the control vermitea (1). However, the differences seen between the vermitea types were not statistically significant.

Pollutant retention in soil

The long-term crude soil vermitea (5) was the most effective in soil washing, retaining 16.4% less oil than control vermitea (1) (Fig. 2). There were significant differences in the amount of oil retained in the soil between the vermitea types (Kruskal-Wallis, $H_5 = 50.4115$, $p < 0.001$). Within the groups, the long-term crude soil vermitea (5) was statistically more effective than vermiteas 1, 2, and 4 (Figure 2). The water negative control and vermitea 4 were statistically similar, and both left significantly higher amounts of residual oil in the soil samples in comparison to the four remaining vermiteas.

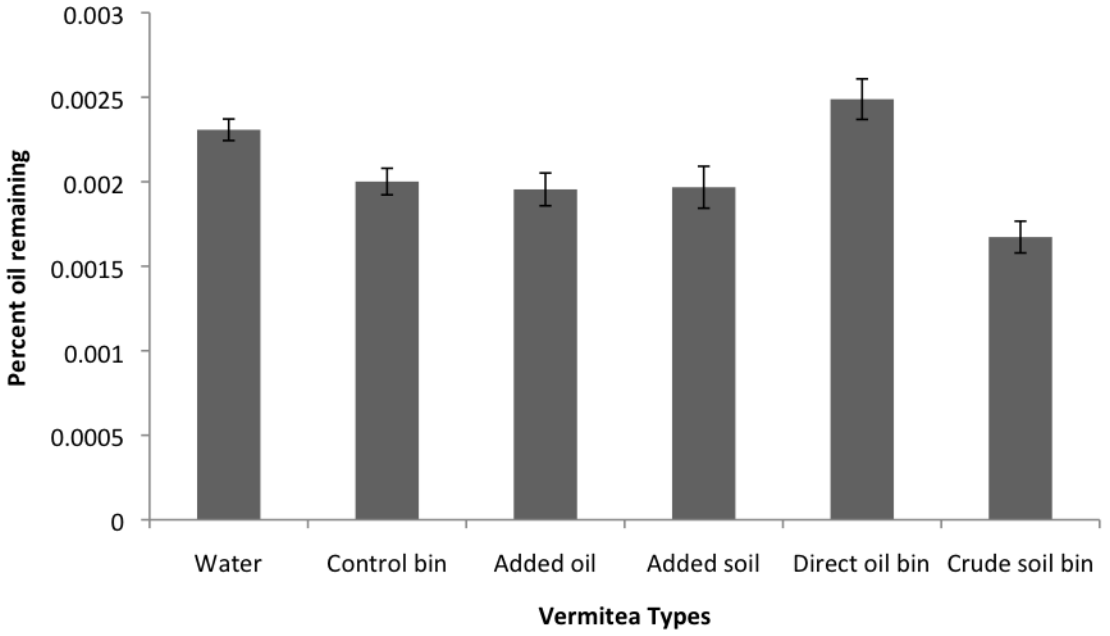

Figure 2. Residual oil in soil. Comparison of soil washing ability between the vermiteas. Between the vermitea types, the long-term crude soil vermitea (5) was significantly more effective in reducing residual oil left in the soil after washing in comparison to the vermiteas not exposed to crude soil ($p<0.001$).

DISCUSSION

Due to the potential ecological risks from current commercial remediation tools (Jain et al 2011), this study aimed to research the effect of prior pollutant exposure on hydrocarbon degradation capacities of various worm compost teas. In order to understand the effect of exposure on bacteria, I conducted physical experiments and community assessments of different vermitea treatments. My findings provided evidence for the remedial abilities of microbial

communities with previous exposure to hydrocarbons in weathered soil, consistent with earlier acclimation studies (Francy et al 1991, Greenwood et al 2009). However, I found that the association between abundance of hydrocarbon degraders and remediation did not hold true on a short timescale, contrary to studies concentrated on the microorganism level of performance (Lin et al 2009).

Emulsification ability of vermiteas

The significant increase in emulsion production in the vermitea prepared with a long-term crude soil exposure (vermitea 5) in comparison to the water negative control suggests that the worm intestine microbial populations in the soil bin were more adapted to hydrocarbon molecules. This finding is contrary to my hypothesis of overall higher emulsification activity in the long-term oil vermitea (4) due to direct pollutant exposure. The high emulsion in the long-term soil vermitea (5) may be a consequence of the mode of oil contamination. Indirectly, the direct exposure of oil may have negatively impacted the worm intestinal microbiota through the adverse effects of the oil towards the worm populations. In comparison, weathered soil may have allowed for a less direct exposure of worms to oil (Semple et al 2003), sustaining healthier levels of worms as seen qualitatively in the crude soil exposed bin, and therefore supporting higher numbers of bacteria overall. I also qualitatively noticed that during three weeks of the feeding period, the direct oil bin displayed noticeably more moist conditions than the control and crude soil bins. As a result of the water retention in the oil bin, the oil may have been less bioavailable to the microbial populations (Roy et al 2003). The lower bioavailability of oil may have led to lower rates of emulsion by microbial populations from the oil bin (Churchill et al 1995). As seen in a previous study, emulsification is correlated with the overall growth of microbial populations in addition to rates of substrate digestion (Ilori et al 2005).

Biosurfactant colony abundances

The significant abundance of biosurfactant-producing colonies in the long-term oil vermitea (4) in comparison to the control is indicative of a community shift in microbial structure. This finding suggests that the oil bin microbial population had a higher overall

abundance of hydrocarbon-degrading bacteria, as predicted in my initial hypothesis. The presence of biosurfactant-producing colonies may be attributed to the selective influence of crude oil for hydrocarbon-degrading bacteria (Kostka et al 2011; Hazen et al 2010) even in the context of the negative effect against the worm population.

The higher number of biosurfactant producers in the long-term oil vermitea samples (4) relative to the short and long-term crude soil vermitea samples (3, 5) conflicts with the relative emulsification values found between the treatments. These results also contradict the well-established association between biosurfactant and emulsion production in individual microbial isolates (Rocha et al 1992, Bognolo et al 1999). The lower number of biosurfactant producers in the contaminated soil vermiteas may be due to the agar medium acting as a filter for the types of visible colonies (Lederberg and Lederberg 1952). In addition, the use of the halo test as a positive indicator for biosurfactant production ultimately did not guarantee the presence of biosurfactant molecules; with the actual identification of the surfactant molecules produced, I would be able to provide better classifications between the bacterial types. The differences between emulsification and biosurfactant colony abundance may also be due to differences between absolute growth rates of the bacteria in the vermitea types. In order to better reconcile the discrepancies seen between emulsification and biosurfactant production, future research should be conducted on the emulsification abilities of the colonies that tested positive for biosurfactant production from both the direct oil and crude soil long-term vermiteas (4, 5).

Relative colony abundance and colony diversity

The similar community abundances and diversity indices between the five vermitea types are indicative of no significant changes in the population numbers and overall morphospecies structure of the microbial communities in the vermitea samples. However, the types of bacteria that make up the communities may have shifted towards those more adapted to the hydrocarbon substrate (Hazen et al 2010; MacNaughton et al 1999). While the results from the vermitea plating were able to give a rough sketch of the changes in absolute population size and diversity, I am limited in my assessment of the bacterial communities in the different vermitea treatments and their associated relative functionalities. With a more in-depth technology, such as a microarray analysis, I could identify the specific taxa populating the treatment samples and

isolate relative abundance differences not detected by simple plating. For future studies on vermitea, conducting Phylochip analyses would contribute in understanding more about the relationship between the community makeup and structure and remediation efficacy.

Pollutant retention in soil

The significant decrease of hydrocarbons in the soil samples from the application of long-term crude soil bin vermitea (5) suggests a higher level of overall microbial consumption activity with respect to the other vermiteas tested. This result may be due to the pre-exposure of the crude soil bin microbial community to the oil substrate in a soil medium. Acclimating the bacteria to feeding from a soil matrix may have allowed for a greater microbial response when exposed to the contaminated soil samples during the washing (Greenwood et al 2009). This finding supports an association between the emulsification activity and the amount of oil degradation observed in the short and long term soil vermiteas. As seen in previous research, the emulsification of oil allows for faster rates of microbial digestion due to increased bioavailability of the pollutant molecules (Banat 1995). However, despite predicting that a higher abundance of biosurfactant-producing microbial strains would be associated with greater oil degradation, this relationship was shown to be not true in the short-term 24-hour soil washing study. As stated earlier, this may be due to the impact of the direct addition of oil in the oil contaminated bin, or the water retention that occurred during the compost-processing period. The length of time the soil was weathered in the oven and the length of exposing the contaminated soil to the vermitea samples may have also affected the relative amounts of oil degradation. With longer oven incubation times to produce a more weathered soil texture, the contaminated samples would have more accurately tested the degradation abilities of the vermitea types (Urum et al 2004). In addition, a longer exposure period of the contaminated soil to the vermitea treatments may have been more faithful to true remediation conditions (Rahman et al 2002). The artificially contaminated soil may have been inconsistently polluted; due to the low concentration of oil used, minute variability would translate to larger relative differences between the treatments. Future studies should broaden the time scale of the experiment with soil from contaminated sites and utilize higher concentrations of oil contamination in the soil being remediated. In my soil washing experiment, I did not analyze pollutant concentrations in the waste leachate removed from the

soil samples after the settling period; this prevents me from concluding how the in situ application of vermitea and eventual runoff from soil sites will affect the surrounding ecosystem.

Limitations and Future directions

Future research should be conducted on the emulsifying qualities of the soil bin vermiteas, and on reconciling the differences between the short and long term exposures to contaminated soil when preparing the vermiteas. In future studies, longer experiments should be maintained to chart the effects of the vermitea application over time and to understand long-term changes in the microbial population. Moving away from artificial soil washing, vermitea can be also applied to small-scale soil models, with irrigation and tilling to further stimulate microbial activity and mimic natural remediation conditions (Straube et al 2003).

In order to develop a more effective vermitea without introducing known pollutants into the ecosystem, it would be beneficial to study how to maximize the effectiveness of the short-term soil-exposed vermitea. During the brewing of the vermitea, exposing microbial populations to the soil from the contamination site intended for remediation would not only prevent more contamination of the site in question, but also potentially increase the digestion activity of the bacteria (Greenwood et al 2009).

Broader implications and conclusions

From this study, I have been able to introduce the use of vermitea as a remediation tool in the context of oil contaminated soils. Through the expansion of experimental studies, site-specific vermitea could be applied in the bioremediation of contaminated soils on a mainstream scale. As shown in general biosurfactant and microbial community enrichment studies, bioremediation allows for the more natural elimination of pollutants in addition to being relatively inexpensive in comparison to its industrial counterparts (Bognolo et al 1999).

The application of the long-term soil exposed worm casting tea significantly reduced levels of oil contamination in lightly weathered soils. Through the combination of multiple physical and biological tests, I was able to form a greater understanding of the different vermiteas and the microbial diversity associated with them. From the results of these

experiments, the research area of bioremediation can broaden the approaches that are taken in restoring polluted ecosystems.

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APPENDIX A: Soil washing

Table A1: Summary of soil washing conditions used.

Vermitea (mL)	Shaking speed (strokes/min	Shaking time
2	50	10 minutes
2	50	20 minutes
2	50	30 minutes
2	100	10 minutes
2	100	20 minutes
2	100	30 minutes
2	200	10 minutes
2	200	20 minutes
2	200	30 minutes
4	50	10 minutes
4	50	20 minutes
4	50	30 minutes
4	100	10 minutes
4	100	20 minutes
4	100	30 minutes
4	200	10 minutes
4	200	20 minutes
4	200	30 minutes