Paper 3B-03, in: A.R. Gavaskar and A.S.C. Chen (Eds.), *Remediation of Chlorinated and Recalcitrant Compounds—2004.* Proceedings of the Fourth International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey, CA; May 2004). ISBN 1-57477-145-0, published by Battelle Press, Columbus, OH, www.battelle.org/bookstore.

BIODEGRADABILITY AND TOXICITY OF HYDROCARBON LEACHATE FROM LAND TREATMENT UNITS

Sandy L. Scott and Yarrow M. Nelson (ynelson@calpoly.edu)
(California Polytechnic State University, San Luis Obispo, CA)

ABSTRACT: The biodegradability of leachate from the land treatment of hydrocarbon-contaminated soil was investigated in the laboratory using respirometry and toxicity testing in combination with total petroleum hydrocarbon (TPH) measurements. Soil in land treatment units (LTU) had been contaminated with a diesel-like hydrocarbon mixture formerly used as a diluent for crude oil at an oil field in California. Leachate was collected from two different LTUs for treatability testing in a respirometer under aerobic conditions. Only about 12% reduction in TPH concentration was observed after aeration for 161 days, indicating limited biodegradability of the hydrocarbon constituents in the leachate. Similarly, Microtox® toxicity did not change after 130 days. Leachate biodegradability was further tested by comparison to diluent-contaminated groundwater from the same site. Leachate diluted to the same TPH concentration as the contaminated groundwater was three times less toxic, but was much less biodegradable. The recalcitrance of the leachate hydrocarbons may be attributable to their high molecular weight, since the majority of the TPH was long-chained hydrocarbons of C_{20} or greater for leachate. In contrast, the diluent contaminated groundwater has a majority of its TPH concentration in short-chained hydrocarbons of C_{20} or less, which were more easily biodegraded. These short chain hydrocarbons are typically more toxic than the longer chain hydrocarbons, which would explain the observed decrease in toxicity of the diluent-contaminated groundwater during biodegradation.

INTRODUCTION

Soil at the former Guadalupe Oil Field was contaminated with a diesel-range hydrocarbon mixture that was used as a diluent for facilitating pumping the viscous crude oil at the site. Soil from heavily contaminated sites near the ocean has been excavated and options are currently being explored for treatment and/or disposal of this soil. One important option is on-site biological treatment using land farming. Pilot-scale land treatment units (LTU) have been operated on-site to test this option of soil treatment. These pilot studies indicate that the leachate from the LTU contains hydrocarbon contaminants that might have a detrimental effect on the groundwater. Thus it is important to understand the fate, transport and toxicity of this leachate. The purpose of this research is to determine the biodegradability of leachate from pilot-scale LTUs and to determine if the leachate toxicity is reduced by biodegradation.

The composition of the diluent contamination at the Guadalupe site has been described by Haddad and Stout (1996). Approximately 90% of the diluent at Guadalupe is comprised of hydrocarbons with an equivalent carbon range of 14 to 30. Forty-one different polycyclic aromatic hydrocarbons (PAHs) have been identified, and the dominant family of PAHs in the diluent are naphthalenes.

The leachate from a LTU was previously shown to have measurable toxicity (CH2M Hill, 2001 and Coffey 2002). The Microtox® test indicated EC_{50} values of 3.3 to 6.1%. LTU leachate was found to be highly polar (approximately 74%–100% polar) after about 100 days of tilling, watering and nutrient addition (Coffey, 2002). These polar compounds could contribute to leachate toxicity. PAHs are also toxic (Kropp and Fedorak, 1998), and may contribute to the toxicity of the leachate.

Leachate biodegradability and toxicity were investigated in two laboratory treatability experiments. In the first experiment biodegradability and changes in toxicity were determined for leachate from a land treatment unit (LTU3) with soil exhibiting high total petroleum hydrocarbon (TPH) concentrations. Biodegradability was determined by measuring respiration rates in a respirometer and by measuring TPH concentrations initially and at 51 days and 161 days. The second experiment was used to compare the biodegradability and toxicity of leachate with that of diluent-contaminated groundwater of similar TPH concentration. For this second experiment leachate from Land Treatment Unit 2 (LTU2) was used, which had significantly lower soil TPH concentrations than LTU3. Toxicity in both experiments was estimated using the Microtox® test.

MATERIALS AND METHODS

The leachate for Experiment 1 was collected from lysimeters on LTU3. The test plot at LTU3 had been tilled on a bi-weekly basis and nutrients had been added. The leachate for Experiment 2 was generated by leaching fresh water through diluent-contaminated soil from Land Treatment Unit 2 (LTU2). Samples from each LTU site were incubated at 25°C under controlled laboratory conditions while measuring either O_2 consumption or CO_2 production with a respirometer. Microtox® toxicity was determined for initial samples and periodically during biodegradation in duplicate or triplicate to determine if the toxicity was decreasing. The TPH concentration of the leachate was analyzed for initial and final samples using gas chromatography/mass spectroscopy (GC/MS) by Zymax Envirotechnology to determine if the hydrocarbons were biodegrading.

For Experiment 1 with LTU3 leachate, respiration was measured for triplicate 2-L samples of leachate and one control of San Luis Obispo tap water. Two liters of sample were used to provide two 1-L samples for TPH analyses at 51 days and 161 days. No nutrients were added for this test to observe biodegradation in an unamended state.

In Experiment 2 biodegradation and toxicity of leachate was compared to that of diluent-contaminated groundwater. Leachate with 24 mg/L TPH was diluted to have the same initial TPH concentration as the comparison diluent-contaminated groundwater samples. Inoculum was not added to either the leachate or groundwater samples during this experiment. Nutrients were added to both the leachate and contaminated groundwater to ensure adequate nutrient availability.

The Microtox® test (Strategic Diagnostics Inc., Newark DE) was used for all toxicity assays in these experiments. The Microtox® test software calculates the results of the test as EC_{50}. The Microtox® EC_{50} is the effective concentration for which 50% of the bioluminescence of the test bacterium *(Vibrio fischeri)* is extinguished by toxicity.

RESULTS AND DISCUSSION

Biodegradability and Toxicity of Leachate from LTU3. The initial TPH concentration of the triplicate leachate samples was 96 ±4 mg/L (Table 1). Hydrocarbon biodegradation

TABLE 1. LTU3 leachate TPH degradation.

	TPH Concentration (mg/L)			Percent TPH Degraded (161 days)
	Day 0	Day 51	Day 161	
Sample 1	98	74.9	72	26.53 %
Sample 2	92	98.9	86	6.52 %
Sample 3	100	89	96	4.00 %

was very slow for the 161 days of this experiment. The average TPH degradation was 12% and the standard deviation was ±12%. The majority of TPH biodegradation appears to have been for the C-18 to C-24 range (data not shown). No decrease in Microtox® toxicity was observed with for the 130 days (Figure 2). Initially EC_{50} for the leachate was 9%. Oxygen uptake was significant after an initial 10-day lag phase (Figure 1). The very high TPH values in LTU3 leachates are indicative of separate phase product in the sample.

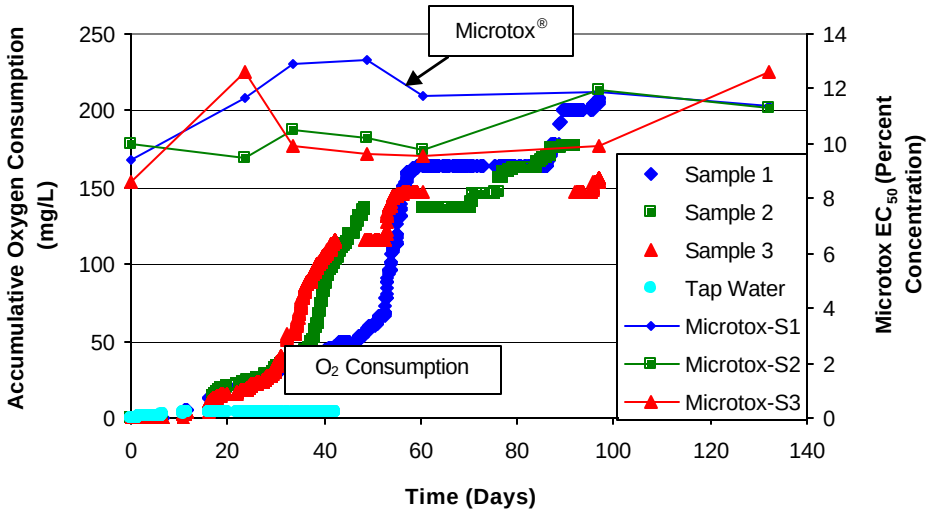

FIGURE 1. Cumulative oxygen consumption and Microtox® toxicity for triplicate samples of leachate from LTU3 during Experiment 1.

There are several possible reasons the LTU3 leachate was only partially biodegraded and its toxicity did not decrease during Experiment 1. First, the easily biodegraded hydrocarbons in the contaminated soil may have already biodegraded during LTU3 operation, leaving the more recalcitrant hydrocarbons as residual. Second, the leachate may have been nutrient-limited since ortho phosphate was non-detect in the leachate samples. Nutrients were added in the second leachate experiment to address this possibility. Third, the leachate used in Experiment 1 might not be representative of typical leachate from LTU3 because the TPH concentration of this leachate sample was much higher than usual. This suggests that the sample may have contained some separate-phase

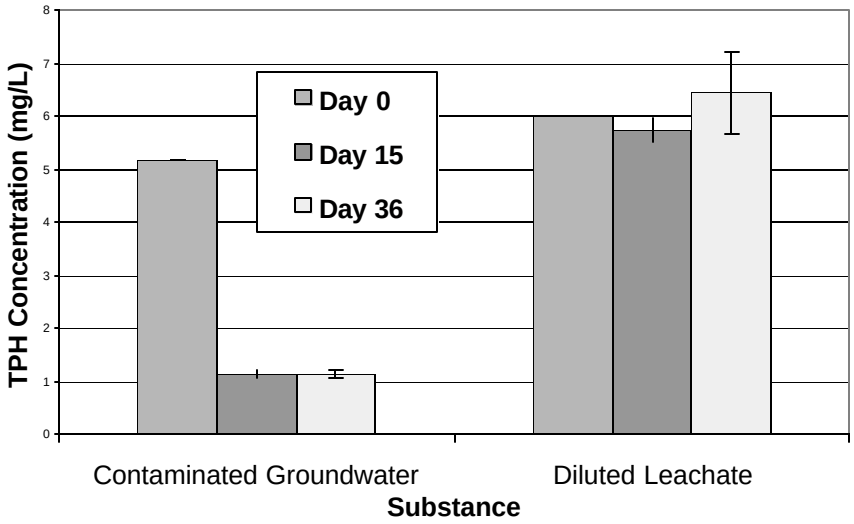

FIGURE 2. Biodegradability comparison of contaminated groundwater with diluted leachate from LTU2.

hydrocarbons. If so, its composition would not be representative of dissolved-phase leachate. It is also possible that the leachate sample used in Experiment 1 had an unusually high TPH concentration because the sample was collected from lysimeters that had been stagnant over the summer. To avoid this problem in Experiment 2, fresh leachate was collected by running fresh water through the soil prior to collection.

Comparison of Leachate and Diluent-Contaminated Groundwater. Initial TPH concentrations of triplicate leachate samples were 24 mg/L compared to only 5.2 mg/L for the contaminated groundwater. Thus leachate samples were diluted by a factor of 4.5 with clean groundwater to match TPH concentrations. TPH was not detected in the clean groundwater samples used for dilution and controls. Both the leachate and the diluent-contaminated groundwater collected for Experiment 2 were very low in nutrient concentrations, so nutrients were added to both the leachate and contaminated groundwater during the experiment. Nutrients were added as Miracle Grow® Miracid 30.10.10, every 2 weeks of the experiment.

The contaminated groundwater decreased in TPH concentration by about 78%, whereas the diluted leachate showed no significant decrease in TPH concentration in 36 days (Figure 2). These results clearly show that TPH in diluent-contaminated groundwater degrades rapidly while TPH in diluted leachate was not degraded in 36 days (Figure 2).

Respiration was measured for 46 hours each week for 5 weeks. During the first week the diluent-contaminated groundwater (CGW) respired at a much higher rate than the diluted leachate (Figure 3). Respiration rates for the tap water sample were negligible as expected. The clean groundwater sample respired at a rate 5 times lower than the diluent-contaminated groundwater, indicating that observed respiration for the contaminated samples is indeed originating from hydrocarbon biodegradation. The leachate respiration rate was similar to that of the clean groundwater control (Figure 3), indicating little

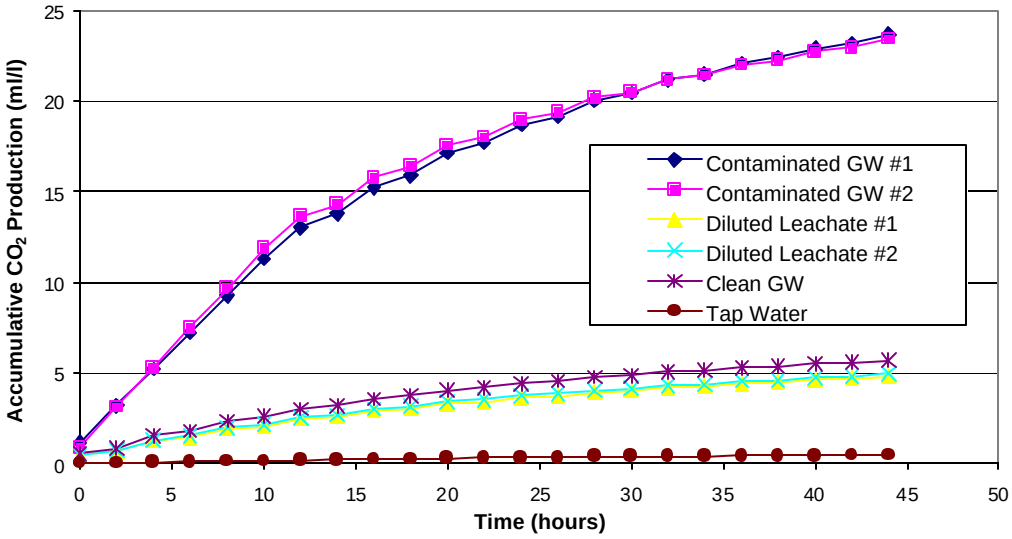

FIGURE 3. Leachate accumulative CO_2 production from LTU2 compared to clean and contaminated groundwater – Week 1.

or no respiration of hydrocarbons in leachate (since the leachate was diluted with this control groundwater). After the first week the respiration rate of the diluted leachate sample began to increase (data not shown). The respiration rate of the contaminated groundwater decreased to levels similar to that of the diluted leachate.

The low biodegradability of the leachate compared to diluent-contaminated groundwater could be caused by differences in their hydrocarbon compositions. Both the diluent-contaminated groundwater and the leachate are composed of complex mixtures of polar organic compounds and aromatic hydrocarbons. Since both types of samples have weathered in the field, it is likely that the alkanes have long-since biodegraded. Further detailed chemical analyses are needed to attribute differences in biodegradability to differences in composition. In the present study the GC analysis was used to quantify equivalent carbon ranges of residual hydrocarbons based on volatility alone (simulated distillation). This analysis indicates that the diluent contaminated groundwater contains lower boiling hydrocarbons, whereas the leachate has more high boiling hydrocarbons (Figure 4). In fact, for the leachate 88% of the TPH is above an equivalent chain length of C_{20} and for the diluent contaminated groundwater 64% of TPH is below an equivalent chain length of C_{20}. It is possible that the low-boiling hydrocarbons are more bioavailable and therefore likely to biodegrade quicker than the high-boiling hydrocarbons. However, further research with more detailed chemical analyses is needed to verify this effect. While these results indicate that hydrocarbon biodegradation in the leachate is very slow, the limited bioavailability of the high-boiling hydrocarbons could also reduce their toxicity, as described below.

The Microtox® toxicity of diluent-contaminated groundwater and leachate diluted to the same TPH concentration as this groundwater are compared in Table 2. The EC_{50} values given in Table 2 are the percent solution that cause a 50% reduction in microbial activity of the test organism (*Vibrio fischeri).* So for example the EC_{50} value of 32.6% observed for the diluent-contaminated groundwater at Day 0 means that a 32.6% solution

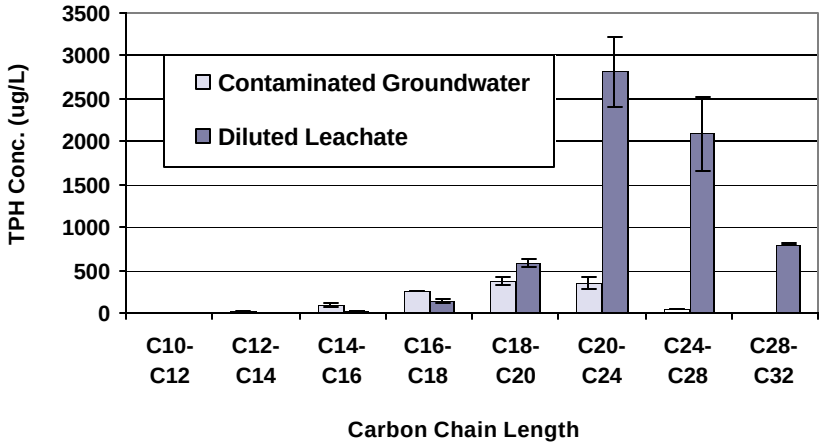

FIGURE 4. Comparison of carbon chain length distribution of contaminated groundwater and diluted leachate from LTU2 after biodegradation for 36 days.

TABLE 2. Comparison of Microtox® toxicity of diluted LTU2 leachate and contaminated groundwater with matched TPH concentrations.

Test	EC_{50} (%) - 5 min.	% Effect at full strength
4/15/03 - Day 0		
Contaminated GW1	32.6	NA
Contaminated GW2	Not tested	Not tested
Diluted Leachate 1	EC_{50} > 100%	4.9
Diluted Leachate 2	Not tested	Not tested
4/30/03 - Day 15		
Contaminated GW1	EC_{50} > 100%	8.9
Contaminated GW2	EC_{50} > 100%	12.2
Diluted Leachate 1	EC_{50} > 100%	21.7
Diluted Leachate 2	EC_{50} > 100%	10.8

of this groundwater caused a 50% reduction of microbial activity. In contrast, the diluted leachate sample did not cause a 50% reduction in activity, even at full strength, so the EC_{50} value for the diluted leachate is greater than 100%. This shows that when the leachate is diluted to have the same TPH concentration as the diluent-contaminated groundwater sample, the leachate toxicity is much less than that of the groundwater. As an additional method of quantifying toxicity, the % effect was calculated by extrapolating to determine the % inhibition of test organism at full sample strength. The % effect values are also given in Table 2. Based on the estimated percent effect at full strength only 5% of the *Vibrio fischeri* were inhibited by the undegraded, diluted leachate at full strength compared to 50% when diluted, like for an EC_{50} (Table 2). So since full strength leachate inhibited by only 5% while a 32.6% solution of the groundwater inhibited by 50%,

leachate diluted to the same TPH concentration as the groundwater is about thirty times less toxic than the groundwater.

After 15 days of biodegradation, the diluent-contaminated groundwater toxicity was reduced to a toxicity with EC_{50} > 100% (Table 2, Day 15). Based on % effect estimated for full-strength samples, the toxicity was only slightly greater for the diluent-contaminated groundwater than for the diluted leachate after 15 days (Table 2). These results suggest that the easily-biodegraded, toxic, components in the groundwater were readily biodegraded in 15 days. To further investigate possible toxic residual compounds, more detailed analysis of the hydrocarbon constituents in both leachate and contaminated groundwater would need to be conducted.

CONCLUSIONS

Only minimal (12%) biodegradation of TPH in LTU3 leachate was observed over the 161 days of Experiment 1. During this time, little or no decrease some N-nutrients but no detectable orthophosphate. However, leachate biodegradation was also very slow in the second experiment during which sufficient N, P and K nutrients were added. It should be noted that in an earlier field study the toxicity of the LTU3 leachate decreased significantly over the course of one year (Coffey, 2002). This suggests that the time frame for leachate biodegradation is just very long.

Similar to the results for LTU3 leachate, little or no TPH biodegradation was observed for the leachate from LTU2 after 36 days. In contrast, biodegradation of TPH in the diluent-contaminated groundwater sample was rapid, with 78% degradation observed in 15 days. Simulated distillations of the TPH analyses indicate that leachate contains organic compounds of higher equivalent carbon number that appear to be much more recalcitrant than the compounds of lower equivalent carbon number in diluent-contaminated groundwater.

The LTU2 leachate toxicity was ten to thirty times less than that of diluent-contaminated groundwater when the leachate sample was diluted to the same TPH concentration. It appears likely that the high-boiling hydrocarbons in the leachate have limited bioavailability and thus have both low biodegradation rates and low toxicity relative to fresh diluent with shorter chain hydrocarbons. This suggests that TPH concentration alone may not be an appropriate indicator for remediation endpoints, and that bioavailability of residual TPH should also be considered.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the direction provide by Paul Lundegard (Unocal). The authors also thank Bob Pease (LFR), Bunkim Chokshi (Cal Poly) and Patricia Coffey (Cal Poly) for technical assistance. Funding for this study was provided by the Unocal Corporation.

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