

TOXICITY TESTING TO EVALUATE BIOREMEDIATION ENDPOINTS AT UPSTREAM OIL AND GAS FACILITIES

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INTRODUCTION

The Canadian Council of Ministers of the Environment (CCME) recently developed (June 2000) standards for petroleum hydrocarbon (PHC) contamination in soil. Alberta Environment (AENV) subsequently adopted the CCME PHC standards within the AENV Soil and Water Quality Guidelines for Hydrocarbons at Upstream Oil and Gas Facilities (AENV, 2001). The CCME PHC standards designate four hydrocarbon fractions (F): F1:C₆-C₁₀, F2:>C₁₀-C₁₆, F3:>C₁₆-C₃₄ and F4:>C₃₄-C₅₀. These fractions have their own associated criteria based upon the likelihood that constituents within a given fraction could produce a potential environmental or human health risk. The rationale being those hydrocarbon fractions that may pose a greater risk (*i.e.* C₆-C₁₀ and >C₁₀-C₁₆) are more highly regulated. The CCME standards also take into account soil texture (*i.e.*, fine- versus coarse-grained), soil location (*i.e.*, surface soil versus subsoil), possible land uses (*i.e.*, agricultural, residential, commercial and industrial) and potential exposure pathways (*i.e.*, soil ingestion, vapor inhalation and eco-soil contact *etc.*). The CCME PHC standards for ecosoil contact pathway are based substantially on toxicity tests conducted with different carbon fractions of PHC, distilled from a single hydrocarbon mixture (Federated Crude), and then freshly added to pristine soils.

Ex situ bioremediation is a cost-effective remediation strategy for treating PHC contaminated soils and has extensively been utilized at several upstream oil and gas settings within Alberta. Bioremediation is widely used to treat hydrocarbon-contaminated soils; however, following treatment some residual contamination generally remains (Angehrn et al., 1998). Residual hydrocarbon concentrations (particularly PHC F3) often remain in excess of CCME/AENV guidelines following bioremediation. The toxicity of a contaminant in soil is related to bioavailability. Bioavailability of freshly added PHC may not be representative of aged PHC contamination in soil. Recent research has demonstrated that organic compounds that have been aged in the field may have appreciably lower bioavailability than the same compounds freshly added to soil (Alexander, 2000).

The reasons for the plateau in hydrocarbon concentrations during bioremediation are well documented in the literature and are often generalized as the 'hockey stick' effect (Alexander, 1995). The explanation for this observed phenomenon is related to a combination of: 1) sequestration of contaminants within inaccessible pore spaces and voids, 2) rate-limited mass transfer associated with both hydrophobic organic contaminant transfer to the aqueous phase and diffusion of more readily degradable compounds from deeper within the soil matrix to the aqueous phase; and 3) bioavailability issues surrounding the aforementioned physical-chemical limitations in conjunction with a lack of microbial access to amenable organic substrates.

The ongoing refinement of standards requires data relating PHC concentrations in aged samples containing complex mixtures (as opposed to distilled fractions) of hydrocarbons to measured toxicity in soil organisms. CCME has indicated that there remains a known information gap related to the ecotoxicology of weathered PHC (CCME, 2000). A battery of acute and subchronic toxicity studies was initiated in the summer of 2000 to evaluate: 1) the toxicity of residual PHC in four weathered biotreated soils and a pristine control soil; and 2) the AENV/CCME PHC standards against the observed ecotoxicity from weathered hydrocarbon-contaminated soils. The composition of the PHC F3 fraction within the four-biotreated soils was also reviewed and compared to the composition of the distilled PHC F3 fraction used during CCME Standards development.

MATERIALS AND METHODS

Physical and Chemical Characterization of Soils

Hydrocarbon contaminated soil samples were collected from four upstream oil and gas facilities in Alberta, Canada. Soil samples were collected to reflect a range of hydrocarbon concentrations (relative to CCME/AENV guidelines) as well as to represent various potential sources of contamination on upstream facilities (e.g., wellheads, drilling sumps etc). Samples were collected from each land treatment area, once a plateau in hydrocarbon degradation was observed and further reductions in PHC levels seemed unlikely. Residual PHC concentrations were above CCME/AENV guidelines for various soil types, land uses and exposure pathways.

The origin of contamination for Soils A, C, and D was from sump material collected at three different sites, while the source of contamination for Soil B was from a wellhead release. In general sump material contains contamination that includes crude oil and drilling wastes, while wellhead contamination contains only crude oil. Hydrocarbon characterization revealed that PHC within all four soils generally had a carbon distribution between C₁₅-C₄₀. The biotreated material

contained no detectable volatile hydrocarbon compounds (C₆-C₁₀) corresponding to the CCME PHC F1 fraction. Additionally, it is assumed that the majority of water-soluble organic compounds would have degraded, volatilized, or washed out of the soil profile during treatment.

Appropriate control soils were not available at or near the contaminated sites so a pristine control soil was selected based on correlative soil variables. The main variables used for selection of the pristine soil were pH, electrical conductivity (EC), texture, and bulk density.

Soil samples were collected from each of the four land treatment areas and then taken to the lab and prepared for chemical and physical analyses. All collected samples were air dried at 50 °C for 18 hours ± 2 hours. Each sample was then ground to a maximum 2 mm aggregate size using a manual grinder. Samples from each site were combined to create one large site composite sample, which was stored in a 1-gallon pail. Each site composite sample was homogenized by stirring with a wooden stick and by rolling the pail on the ground for 10 minutes. Following homogenization, each site composite sample was then split into three subsamples. Analysis for particle size distribution within the subsamples was used to evaluate the relative homogeneity of each subsample prior to subsequent inclusion in characterization and/or experimental trials.

Soils were analyzed for PHC, polycyclic aromatic hydrocarbons (PAHs), pH, EC, and particle size. PHC concentrations were determined using protocols outlined by the CCME (CCME, 2000). Briefly, quantification of PHC fraction F1 involves a methanol extraction and purge and trap procedure followed by GC/FID. Fractions F2, F3 and F4 (up to C₅₀) were first extracted using a Soxhlet extraction (50:50 hexane:acetone) procedure. The extract was then dried using sodium sulfate and treated with silica gel (to remove polar materials). The extract was then analyzed using GC/FID analysis. For the C₅₀₊ fraction, either a gravimetric or extended high-temperature chromatography determination is made (CCME, 2000). For the purposes of this study, historical data (not presented) suggested that characterization of the PHC F1 (C₆-C₁₀) fraction was not necessary. The PHC F1 fraction is the most volatile and easily degraded, and since these soils were biotreated in the field for several years, sufficient opportunity was provided for the volatile components to degrade/dissipate. Soil PAH analysis followed EPA 3545, EPA 8270, and EPA 3611B procedures. Soil chemical analyses were conducted in triplicate and values are reported as the average of replicate samples (result) ± 95 % confidence interval.

Toxicity Testing Protocols

Toxicity bioassays were conducted in triplicate and consisted of: Microtox™, radish (*Raphanus sativus*) and oat (*Avena sativa*) seed germination; as well as

acute (14 day) and subchronic (10 week) exposures of the earthworm (*Eisenia fetida*).

The Microtox™ toxicity assay was conducted on 1:1 water extracts from the four-biotreated soils according to procedures outlined by Environment Canada (Environment Canada, 1992). The microbial toxicity assay was conducted in triplicate for each contaminated soil.

A seed germination bioassay was conducted for each contaminated soil as well as for the control soil. Radish (*Raphanus sativus*) and oats (*Avena sativa*) were selected for seed germination trials. The first exposure was to 100 % contaminated material, the second treatment was a 50 % dilution of the contaminated material with a pristine control soil (w/w), and the final treatment was a 100 % pristine control soil. The germination assay followed the principles of ASTM method E 1598-94 (1994) and was done in triplicate.

Earthworm survival was the endpoint used for the toxicity assays described here. The earthworm toxicity bioassay incorporated many of the principles described in ASTM, (1995); and Greene et al (1989). Briefly, the protocol is outlined below. Mature earthworms (*Eisenia fetida*) were used for all acute (14 day) and subchronic (10 weeks) toxicity tests. Soil treatments for the acute and subchronic toxicity testing consisted of the three exposure treatments, conducted concurrently. The first exposure was to 100 % contaminated material, the second treatment was a 50 % dilution (data not presented) of the contaminated material with a pristine control soil (w/w), and the final treatment was a 100 % pristine control soil. Treatments were conducted in triplicate with ten worms being exposed per jar, for a total of thirty earthworms per treatment. Earthworms were placed on the surface of each of the test soils and no physical effort was made to force the worms to burrow into the soil. To monitor survival, the worms were emptied out of the jars and counted daily for the initial 14 days (acute toxicity test period) and weekly thereafter (subchronic toxicity testing period) for the following eight weeks. Soil was returned to the corresponding jar and the live worms were placed back on the surface. The assays were conducted under lighted conditions, to facilitate burrowing into the soil, and at a constant temperature (22 °C).

RESULTS AND DISCUSSION

Physical and Chemical Characterization of Soils

Results from general soil characterization are provided in Table 1. Soil textures according to the Canadian Society of Soil Science (1978) are Clay Loam for Soils A, B and C and Loam for Soil D and the Control Soil. Based on the results of

particle size distribution, all soils (A, B, C and D) are classified as fine textured (greater than 50% by mass < 75 μ m) as defined by CCME.

Results from hydrocarbon characterization within biotreated soils are provided in Table 2. For comparison purposes, AENV/CCME PHC standards for fine textured surface and subsurface soils have also been provided (Table 2). PHC concentrations within all soils are in excess of AENV/CCME PHC standards for various soil types, land uses, and exposure pathways. PHC concentrations for F2 and F4 are below the CCME standards while the PHC F3 fraction remains elevated above CCME PHC surface soil standards in all four soils; assuming the relevant exposure pathway is ecosoil contact. Thus, it is the F3 fraction that causes the soils to fail for PHC under the current AENV/CCME standards.

For comparison purposes, the PHC F3 hydrocarbon composition (separated into C₁₆-C₂₁ and C₂₂-C₃₄ subfractions) from the Federated Crude used in CCME Standards development and Soils A through D (post-biotreatment) are provided below:

Sub-fraction	CCME (Fed Crude)	Soil A	Soil B	Soil C	Soil D
C ₁₆ -C ₂₁	0.46	0.24	0.23	0.21	0.29
C ₂₂ -C ₃₄	0.54	0.76	0.77	0.79	0.71

Significant variation was observed amongst PHC F3 composition within Soils A through D compared to Federated Crude used in CCME Standards development.

PAHs were detected within Soils A, C and D but were below detection (< 0.05 mg/kg) within Soil B. Quantifiable PAHs ranged from 2-ring (naphthalene) to 4-ring compounds (chrysene); however, PAH concentrations in Soils A, C and D were not considered appreciable.

Toxicity Testing

Results from the battery of toxicity tests are presented in Table 3. Results from the Microtox™ assay indicate that all soil extracts were non-toxic (both EC₂₀ and EC₅₀ were > 100 %) throughout the test period.

Seed germination assays indicate that \geq 90 % of both radish and oats had germinated in all soils within four days. A seed germination rate of \geq 90 % was used to indicate that the soils were non-toxic to the plant seeds. Data for 50% diluted soil were not presented as 100 % contaminated soil initiated non-toxic responses in test organisms, as measured, and the 50 % dilution data did not significantly differ from the 100 % treatment.

Results from the earthworm survival bioassay (Table 3) indicate that earthworm survival was 100 % during both the acute (14 day) and chronic (10 week) phases of the experiment. Toxicity data for 50 % diluted soil (not presented) had a 100 % survival rate as well. There was no visible evidence of any behavioral impairment to the earthworms.

Discussion

The development of the CCME PHC standards involved the addition of fresh PHC to soil and the measured toxic response(s) to a variety of organisms at various levels of biological organization. The PHC standards development protocol produced PHC criteria that are protective of ecological health impacts from fresh spills or discharges to soil as this PHC is readily bioavailable and capable of eliciting a toxic response. The AENV/CCME PHC standards do not incorporate the transformation of hydrocarbons due to weathering, interactions amongst various hydrocarbon fractions and the development of bioavailability and mass transfer limitations over time. Further, AENV/CCME PHC standards (ecosoil pathway) were based primarily on the observed toxic response from exposure to a single hydrocarbon mixture (e.g., Federated Crude). As such, the AENV/CCME Tier I standards may be overly-protective of the ecological risk actually posed by residual hydrocarbons in soil following biotreatment, at least for the sites investigated in this study using microbiological, seed germination and earthworm toxicity trials.

CONCLUSIONS

Although residual PHC concentrations remained in excess of AENV/CCME PHC guidelines there was no observed toxicity to microbes, vegetation or earthworms (as measured). Experimental results indicate that the toxicity, and thus the risk, of weathered PHC in biotreated loam to clay loam soils may not be captured by the current AENV PHC guidelines. Results from experimental data also suggest that the PHC mixture (particularly PHC F3) used in the development of CCME standards may not be representative of hydrocarbon mixtures encountered in all upstream oil and gas settings.

In order to determine the actual risk of weathered PHC to various ecological receptors, toxicity tests should be performed on weathered PHC in conjunction with site-specific chemical characterization. The coupling of site-specific toxicity data with chemical characterization should assist in achieving remediation goals and standards that are more appropriate for weathered PHCs and still protective towards environmental risks.

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REFERENCES

Alberta Environment. 2001. Alberta Soil and Water Quality Guidelines for Hydrocarbons at Upstream Oil and Gas Facilities. Draft.

Alexander, M. 1995. "How Toxic are Toxic Chemicals in Soil?" *Environmental Science and Technology*. 29(11): 2713-2717.

Alexander, M. 2000. "Aging, Bioavailability, and Overestimation of Risk from Environmental Pollutants." *Environmental Science and Technology*. 34(20): 4259-4266.

ASTM. 1994. *Standard Practice for Conducting Early Seedling Growth Tests*. E1598-94. American Society for Testing and Materials. West Conshohocken, PA.

ASTM. 1995. *Standard guide for conducting a laboratory soil toxicity test with lumbricid earthworm Eisenia foetida*. E1676-95. American Society for Testing and Materials. West Conshohocken, PA.

Angehrn, D., R. Galli, and J. Zeyer. 1998. "Physicochemical Characterization of Residual Mineral Oil Contaminants in Bioremediated Soil." *Environmental Toxicology and Chemistry*. 17(11): 2168-2175.

Canadian Council of Ministers of the Environment (CCME). 2000. *Canada-Wide Standards for Petroleum Hydrocarbons (PHC) in Soil*. Canadian Council of Ministers of the Environment, Winnipeg, MB.

Canadian Soil Survey Committee, Subcommittee on Soil Classification. 1978. *The Canadian System of Soil Classification*. Canadian Department of Agriculture. Publ. 1646. Supply and Services Canada. Ottawa, ON.

Environment Canada. 1992. *Biological test method: Toxicity test using luminescent bacteria (Photobacterium phosphoreum)*. Environment Canada, Ottawa, Ontario. Report EPS 1/RM/24.

Greene, J.C., C.L. Bartels, W.J. Warren-Hicks, B.R. Parkhurst, G.L., Linder, S.A., Peterson, and W.E. Miller. 1989. *Protocols for short term toxicity screening of hazardous waste sites*. EPA 600/3 - 88 - 029, United States Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.