

Ecotoxicological endpoints for contaminated site remediation

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Summary. - Use of chemical criteria in assessing the potential for adverse toxic effects in contaminated sites can under or overestimate the necessary level of site cleanup required. The use of ecotoxicity testing provides a more direct assessment of adverse environmental impact. A multi-trophic level soil ecotoxicity assessment was done on soil contaminated with crude oil distilled into five different fractions based on hydrocarbon chain lengths. Results indicate that the fraction above C₂₆ was not toxic to microbes, plants, and earthworms, when present in concentrations far above the 1000 mg/kg total petroleum hydrocarbon criterion. Our ecotoxicity test battery results indicate that weathered heavy crude oils can be much less toxic than lighter, freshly spilled diesel oils, yet using a gross measure of total petroleum hydrocarbons would not detect this difference.

Key words: ecotoxicology, contamination, remediation, petroleum, crude oil.

Riassunto (*Endpoints ecotossicologici per il recupero dei siti contaminati*). - L'utilizzo di criteri chimici per stimare gli effetti tossici di siti contaminati può sotto o sovrastimare il livello minimo richiesto di risanamento del sito oggetto di studio. L'uso di test ecotossicologici fornisce una valutazione più diretta sull'impatto ambientale. Una stima del suolo a livello multitrofico è stata eseguita su terreni contaminati da petrolio greggio distillato in cinque frazioni differenti basate sulle lunghezze delle catene idrocarburiche. I risultati indicano che la frazione sopra C₂₆ era atossica ai batteri, alle piante ed agli oligocheti quando presente in concentrazioni superiori ai 1000 mg/kg al test di verifica totale dell'idrocarburo. I nostri test di ecotossicità sembrano indicare che i petroli greggi esposti all'aria possono risultare molto meno tossici di quelli leggeri, tuttavia usando gli idrocarburi totali del petrolio non si rilevarebbe questa differenza.

Parole chiave: ecotossicologia, contaminazione, risanamento, petrolio, petrolio grezzo.

Introduction

The use of generic chemical criteria for contaminated site assessment and remediation goals are limited by their inability to account for site-specific background conditions, the presence of unknown chemicals or mixtures of chemicals in the soil and their potential ecological effect of soil biota. A survey of the most recent chemical criteria for selected metals and organic compounds in Canada and Italy revealed large differences, sometimes by orders of magnitude, between the two countries [1, 2]. Examples of criteria comparisons are shown in Figs 1-3. In particular, the Italian industrial soil criteria for zinc (Zn), arsenic (As), cyanide (CN-) were over an order of magnitude higher than the Canadian or Alberta provincial criteria.

These differences may be due to different regional geology, background soil characteristics, or the most sensitive target species used to define protective chemical concentrations. However, the large differences underscore the uncertainties associated with applying generic chemical criteria to all sites. The use of site-specific criteria, developed using actual ecological

effects data from the site can provide more realistic criteria and minimize unnecessary remediation work or expensive soil excavation and burial in landfills.

Work by the United States Department of Energy (US DOE) have developed preliminary remediation goals based on ecological endpoints specific for their Oak Ridge site [3-5]. They used microbes (heterotrophic processes), plants and invertebrates to establish toxicological benchmarks and then compared them to site-specific background soil concentrations of the contaminants of concern at Oak Ridge and to average soil of the Eastern United States. Examples of the US DOE benchmark chemical criteria for some metals for protection of earthworms are compared to the current Italian criteria in Fig. 4. In most cases the Italian criteria exceed those developed for US DOE at Oak Ridge, however, the Italian criterion for mercury (Hg) was almost two orders of magnitude higher. Again, these differences can reflect the different soil backgrounds and selected target species sensitivity.

The Italian criteria did not list a value for petroleum hydrocarbons, which is more important in the oil-producing province of Alberta in Canada. Recently the

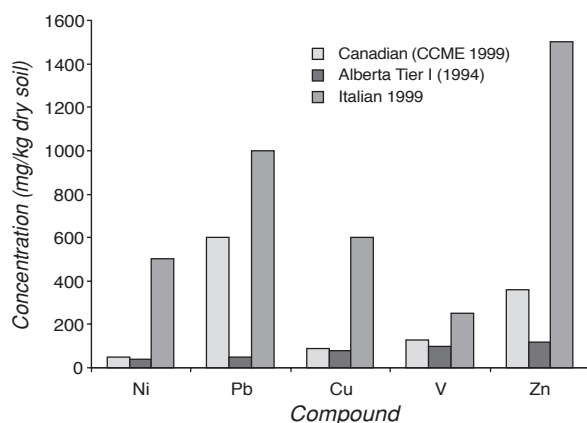


Fig. 1. - Comparison of selected metals. Criteria for industrial use soil.

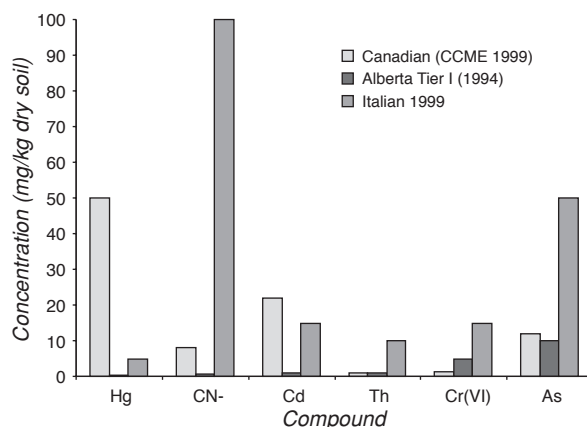


Fig. 2. - Comparison of selected inorganic chemicals. Criteria for industrial use soil.

Canadian Council of Ministers of the Environment (CCME) have developed hydrocarbon criteria based on ecological receptors [6]. Several crude oil contaminated sites have undergone bioremediation to reduce the total petroleum hydrocarbons (PHC) in the soil. Most sites cannot reach the previous remediation criteria of 1000 mg/kg TEH because of the resistance of the heavier hydrocarbon fractions (i.e., > C₂₅) present in unrefined crude oils to biodegradation.

HydroQual Laboratories and the University of Calgary have been working to determine the ecotoxicity of heavier fractions of crude oils, which do not easily biodegrade and may be less bioavailable to soil biota. The results of our study to evaluate the contribution of the heavier hydrocarbon fractions to the total crude oil toxicity in soil ecosystems are presented here.

Methods

Five fractions of paraffinic, asphaltic and naphthenic crudes were prepared by ASTM D2892 simulated steam distillation method to yield fractions with different

carbon content ranges. The relationship between the hydrocarbon boiling point ranges obtained and the average hydrocarbon number in each range is summarized in the Table 1.

Toxicity of aqueous and methanol extracts of each fraction alone or in soil was tested for toxicity to bacterial luminescence and lettuce root elongation. Solid phase tests included earthworm survival and lettuce seed emergence.

Crude oil fraction characterization

Each fraction was analyzed by gas chromatography using an n-paraffin standard to assign carbon number equivalents to each boiling point fraction.

Each fraction was analyzed for the hydrocarbon component classes - saturates, aromatics, polars, and asphaltenes by silica gel column chromatography. Asphaltenes were separated by n-pentane precipitation and filtered for gravimetric determination. The de-

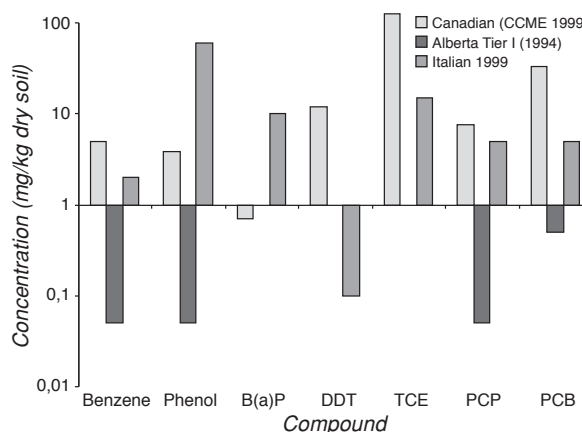


Fig. 3. - Comparison of selected organic compound. Criteria for industrial use soil.

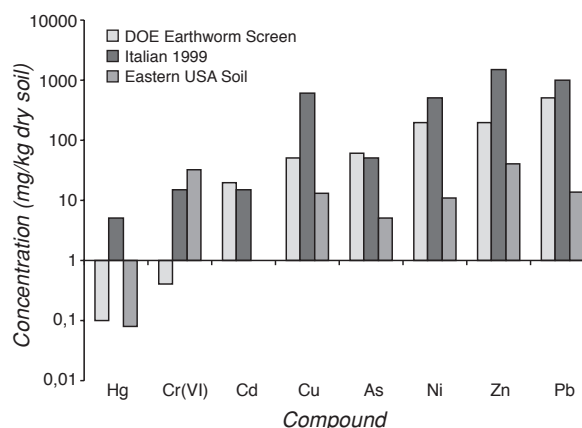


Fig. 4. - Comparison of US DOE earthworm screening benchmarks to organic criteria.

Table 1. - Initial boiling point and carbon range equivalent for paraffinic, asphaltic and naphthenic crudes

Boiling point range	Carbon number
Initial boiling point (IBP) to 205 °C	C ₉ -C ₁₃
206-260°C	C ₁₂ -C ₁₆
260-315°C	C ₁₄ -C ₂₀
315-425°C	C ₁₈ -C ₂₈
> 425 °C	C ₂₆ -C ₄₅

asphalted oil (maltenes) was then eluted through a silica gel column with methylene chloride to elute the saturates, toluene to elute the aromatics, and methanol to elute the polars. All component classes were determined by gravimetric analysis and expressed as a weight percent of each total crude oil fraction. The component classes in naphthenic crude oil are shown in Fig. 5 as an example.

Preparation of extracts

Aqueous and methanol extracts of each crude oil and their distillation fractions were prepared as follows. To prepare standard 4:1 oil: solvent extracts, 5 g of each oil sample and 20 ml of either deionized water or methanol was placed into 40 ml glass vials with Teflon-lined screw cap lids. Extraction was done at 22 °C for 24 h by end-over-end tumbling on a roller. Extracts were recovered as the aqueous or methanol phase and stored at 4 °C.

Preparation of oiled soils

To eliminate the known toxic volatile fractions, each crude oil was first "topped" by heating in a 50 °C water bath for 1 hour while a stream of nitrogen gas was bubbled through the oil.

To assess the impact of crude oil spills on surface topsoil, a clean loam soil from South of Calgary was used to prepare oiled soils. Initial soil moisture content was 15.7 wt%. Bulk density was 1.125 kg/m³. Initial organic carbon content was 3.1 wt%.

Each topped crude oil (500 g) was added to soil at 5 wt% or 50,000 mg/kg concentration, typical of a heavily oil-contaminated soil. The oiled soils were allowed to age at room temperature (18-25 °C) for one year to allow natural biodegradation and sorption events to occur. No active bioremediation by water or fertilizer addition or soil aeration was done to minimize rapid biodegradation of the added crude oils. Soil jars were covered with loose fitting lids to allow some air exchange during ageing.

To assess effect and recovery of the crude oil fractions added to the loam topsoil, each crude oil fraction was added to its weight equivalent in 5 wt% whole crude, based on the weight percent in the whole crude. Soils were only incubated for 24h prior to extraction to

minimize biodegradation effects on the recovered toxicity in extracts. Soil phase tests would allow biodegradation to occur simultaneously. Because of the solid form of the heaviest fraction (> 425 °C boiling point) of the paraffinic and asphaltic crudes, no soil tests could be performed. The naphthenic crude was sufficiently fluid after warming to 50 °C to allow adequate mixing into the soil. These heavy crude fractions were insoluble in methylene chloride and so could not be added as a solution in this solvent.

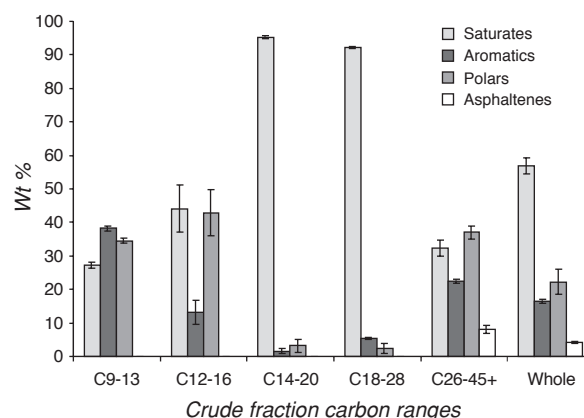
Preparation of oiled soil extracts

To recover aqueous and methanol extracts from oiled soils, 4:1 solvent: soil extracts were prepared by adding 80 ml of solvent to 20 g of soil in 125 ml glass sample jars with Teflon lids. Extraction was done at 22 °C for 24 h by end-over-end tumbling on a roller. Extracts were recovered as supernatants following centrifugation at 3000 x g for 10 minutes. All extracts were stored at 4 °C.

Toxicity testing

The bacterial luminescence test is based on light output by the marine bacterium, *Vibrio fischeri* [7]. Substances that are toxic or stressful will reduce bacterial light output. The aqueous extracts were tested at 91% full strength, whereas the methanol extracts were tested at 5%. Serial dilutions were then tested to determine EC50 values as necessary. Readings were taken after 15 minutes using the Microtox' Model 500 Unit (Azur Corporation). All results were expressed as a percentage of light output in the controls (water or 5% methanol) and IC50 (concentration causing 50% inhibition of light output compared to controls) was calculated.

Root elongation tests were conducted following the procedure of Greene *et al.* [8]. Ten seeds were placed on a Whatman no. 3 filter paper in a 10 cm plastic Petri dish moistened with 4 ml of the extract (water or 1% methanol; highest methanol concentration that has no

**Fig. 5.** - Naphthenic crude oil fractions.

adverse effect on germination and root growth). The dishes were capped with lids, sealed with Parafilm and incubated at 23 ± 2 °C in darkness for five days. Seeds with root tips emerging or with a split seed coat were considered germinated. Lettuce root lengths were measured from the root tip to the base of the shoot. The transition between the root and shoot of lettuce seeds is clearly defined by a sharp bend. The results were expressed as a mean percent of controls.

The seedling emergence tests were based on the procedures of Greene *et al.* [8], American Society of Testing and Materials [9], and Organisation for Economic Co-operation and Development [10]. Lettuce (*Lactuca sativa*), a standard plant species widely used in research and for toxicity assessment of contaminated soils, was used in the tests.

Thirty grams of soil were placed in a 10 cm plastic Petri dish and leveled with light tamping. Twenty seeds were placed on the soil surface, followed by 30 g of a silica sand cap and water to achieve 75 to 85% of the water holding capacity. The dishes were capped with lids, sealed with parafilm and incubated in darkness for three days at 23 °C, then a further two days under an 8 h dark and 16 h light photoperiod (4000 ± 400 lux). The results were expressed as a mean percent of controls.

The worm survival test is a long-term acute lethality test. The test was performed according to the method of Green *et al.* [8]. Ten mature worms (*Eisenia foetida*) were exposed to 200 g of the test soil or suitable dilution in a small plastic cup. Adequate, but not excessive water was added to moisten the soils before adding the worms. The test containers were covered and incubated at room temperature. At the end of 7 and 14 days, the worm survival was scored. The results were expressed as a mean percent of controls.

A toxicity balance was performed for the bacterial luminescence data by first calculating toxic units (TU) as 100 divided by the IC₅₀. The TU in each crude fraction (TU Crude) was calculated by multiplying TU by the percent of each boiling point fraction in the whole crude and then by 5 to correct for the 4:1 extract dilution. The sum of the TU of the hydrocarbon fractions was then expressed as a percent of the TU in the whole crude.

Results and discussion

Bacterial luminescence

The bacterial luminescence toxicity balance for the 4:1 aqueous extracts from the naphthenic crude fractions was 98%, indicating that the toxicity was not lost during fractionation and aqueous extraction of the fractions. The majority of the toxicity was found in the lighter boiling fractions. The heaviest fraction (bp > 425 °C or > C₂₅ carbon range) accounted for only 2.4% of the total toxicity in the naphthenic crude oil (2 of 82 TU in whole crude).

In another example, the bacterial luminescence toxicity balance for the 4:1 methanol extracts from the paraffinic crude fractions was 109%, indicating that the toxicity was not lost during fractionation and aqueous extraction of the fractions. The toxicity was found in all boiling fractions. The heaviest fraction (bp > 425 °C or > C₂₅ carbon range) accounted for only 13% of the total methanol-extractable toxicity in the paraffinic crude oil. Methanol extracts more non-polar hydrocarbons, including saturates and aromatics.

Lettuce root elongation

Aqueous and methanol extracts were tested at 10% and 1% respectively. The paraffinic and asphaltic crudes showed no toxicity. The naphthenic crude showed marginal toxicity (73% of control) for the 10% aqueous extract. For this test, results of 80% of control or higher are considered non-toxic. The naphthenic crude has the highest fraction of lower boiling material, which would be more water-soluble and potentially more toxic. The methanol extract can only be tested at 1% of the original 4:1 extract because the methanol solvent becomes toxic at higher concentrations.

Lettuce seedling emergence

Crude oils were tested in a reference loam soil containing 5.0 or 0.5 wt% (50000 or 5000 mg/kg) crude oil. The seedling emergence data showed that at 0.5% oil in soil only the paraffinic crude showed marginal effect (72% of controls). At 5% oil in soil both the paraffinic and naphthenic crudes were toxic, but the asphaltic crude was not. The toxic effect of the paraffinic crude and 5% level of naphthenic crude was most likely not chemical, but physical because the soil became hydrophobic, indicated by water not readily infiltrating the soil.

The remediation criteria for oil in soil in Alberta is 1000 mg/kg of 0.1%, which is much lower than levels showing no effect for the crude oils tested here. The seedling emergence test is a solid phase test done in soil, which allows for the maximum exposure of the test seeds to the water insoluble organics in the crude oil.

Worm survival

Crude oils were tested at 2.5% (25000 mg/kg) in reference loam soil for the ability of *Eisenia foetida* to survive for 14 days. At 2.5% in soil, both the paraffinic and naphthenic crude oil was toxic, causing 0% survival of the worms. The asphaltic crude was not toxic, with 90% survival observed. The greater concentration of lighter materials in the paraffinic and naphthenic crudes most likely cause the toxic effect as they are much less in the asphaltic crudes.

The heavy fraction of the naphthenic crude (bp > 425 °C, > C₂₅ carbon number) was tested at 2.5% of this fraction in soil and was found to be non toxic (100% survival). The heavy, molasses-like viscosity of this fraction makes it less bioavailable to the worms, unlike the solvent-effect of the lighter fractions on the worm membranes, which causes the worms to dissolve. The heavy fractions of the paraffinic and asphaltic crudes could not be tested because they were solid tars at room temperature and could not be adequately dispersed throughout the loam soil.

Conclusions

Results demonstrate that the C₂₆+ fractions were significantly less toxic than the lighter fractions for all trophic levels tested. These results support raising the residual crude oil TEH above the 1000 mg/kg criteria for sites where the C₂₆+ represent the majority of the residual TEH in the soil.

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REFERENCES

1. Canadian Council of Ministers of the Environment (CCME). *Canadian environmental quality guidelines*. Winnipeg, MB: CCME; 1999. (www.ccme.ca).
2. Alberta Environmental Protection. *Tier I criteria for contaminated soil assessment and remediation*. Edmonton: Alberta Environmental Protection; 1994.
3. Efroymson RA, Will ME, Suter II GW, Wooten AC. *Preliminary remediation goals for ecological endpoints*. Washington DC: US Department of Energy (DOE); 1997. (ES/ER/TM-162/R2).
4. Efroymson RA, Will ME, Suter II GW, Wooten AC. *Toxicological benchmarks for screening contaminants of potential concern for effects on terrestrial plants: 1997 revision*. Washington DC: US Department of Energy (DOE); 1997. (ES/ER/TM-85/R3).
5. Efroymson RA, Will ME, Suter II GW. *Toxicological benchmarks for contaminants of potential concern for effects of soil and litter invertebrates and heterotrophic process: 1997 revision*. Washington DC: US Department of Energy (DOE); 1997. (ES/RE/TM-126/R2).
6. Canadian Council of Ministers of the Environment (CCME). *Petroleum hydrocarbons in soil. Canada Wide Standard (CWS)*. Winnipeg MB: CCME; 2000.
7. Environment Canada. *Biological test method: toxicity test using luminescent bacteria (Vibrio fischeri)*. Ottawa: Environment Canada; 1992. (EPS 1/RM/24).
8. Greene JC, Bartels CL, Warren-Hicks WJ, Parkhurst BR, Linder GL, Peterson SA, Miller WE. *Protocol for short term toxicity screening of hazardous waste sites*. Corvallis OR: USEPA; 1988. (EPA 600/3-88-029).
9. American Society of Testing and Materials. *Standard practice for conducting early seedling growth tests*. West Conshohoken, PA: ASTM; 1996. vol. 11.05. (E1598-94).
10. Organisation for Economic Co-operation and Development. *OECD guidelines for the testing of chemicals, 1993. Method 203. Terrestrial plants growth test*. Paris: OECD; 1993.