Temporal ecological assessment of oil contaminated soils before and after bioremediation

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Abstract

Ecotoxicity methods were used to assess different soil and oil combinations before, during and after laboratory bioremediation with associated hydrocarbon analysis. Heavy, medium and light crude oil (API gravity 14, 30, and 55) was spiked (ca. 5% w/w) into two sandy soils in the laboratory having organic carbon concentrations of 0.3 (Norwood) and 4.7% (Norwood/Baccto). The earthworm (*Eisenia fetida*) 14-d lethality assay, the modified Microbics Microtox® Solid-Phase assay, and the 14-d plant seed germination and growth assays using corn, wheat and oats, were spiked and tested during a 360-d laboratory remediation. *Eisenia* was the most sensitive of the three methods utilized with survival increasing throughout bioremediation with fastest toxicity reduction in the high carbon Norwood/Baccto soils where LC50's were 100% or greater at the end of 90-d whereas, >150-d were required to achieve a similar result in the low carbon soil. Analysis of the undiluted treatments with oily soil alone showed that earthworm survival was high after 90-d in all high organic carbon soils, and after eight months in the low carbon soils, except for the Norwood soil-light oil treatment, which required 360-d to achieve 100% survival. The Microtox assay was less sensitive with EC50's 100% or greater observed after 90-d in high carbon soils and after 240-d for all low carbon soils. After bioremediation, no effects on seed germination were observed, although some plant growth inhibition effects remained. There was no direct correlation between total petroleum hydrocarbon concentrations and toxicity. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

There are numerous challenges to determine the ecological risk of hydrocarbon contamination at waste sites with cleanup decisions averaging $25 million for each site. Efforts to conduct site specific risk assessments using ecotoxicity testing and correlation to contaminant concentration (NOEC) have been very limited and relatively unsuccessful for hydrocarbon contamination. Most of the site ecological assessments have been conducted on large sites where multi-tier testing

and extensive studies have been conducted. The majority of hydrocarbon contamination sites results in small area releases of typically less than five acres. The challenge has been to develop a risk based corrective action (RBCA) approach to incorporate ecological assessment techniques to reach management decisions quickly and for minimal cost (ASTM, 1994). However, little data exists for hydrocarbon contamination effects specific to soil types, oil types and chemically descriptive characteristics in soils with ecotoxicity data. The majority of the hydrocarbon effects data is derived from the use of aquatic data extrapolated to soils considering equilibrium partitioning to estimate bioavailability. Research by numerous workers has

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Table 1
Crude oil characteristics

Origin	Light-Gulf of Mexico	Medium-Gulf of Mexico	Heavy-Southern California
API gravity	53	30	16–18
Saturates (%)	86.7	56.4	20.3
Aromatics (%)	6.4	23.7	28.9
Polars (%)	0.7	14	44.1
Benzene (ppm)	5380	1430	95
Toluene (ppm)	14 000	4590	520
Ethylbenzene (ppm)	1810	1200	370
p,m-Xylenes (ppm)	10 100	6030	390
o-Xylenes (ppm)	4810	1890	360

determined that the hydrocarbon weathering process renders the majority of the hydrocarbon neither bioavailable nor subject to transport (Linz and Nakles, 1997). An ongoing program in our laboratory has been the investigation of invertebrate, plant, and bacterial bioassays to predict response to soils spiked with oil (Dorn et al., 1998) and those from hydrocarbon contaminated sites. Testing has examined responses to freshly oiled soil (types) and those bioremediated for up to one year (Salanitro et al., 1997). In that study Salanitro et al. (1997) investigated the loss of hydrocarbon components in oiled soils of two types to make predictions of remediation. A second aspect of that study was to describe the ecotoxicological responses of plants, microbes and invertebrates to changes in soil conditions resulting from remediation efforts over a 12-month period. Our intent was to develop screening methods for predicting ecological effects at sites and implementation of direct toxicity assessment for remediation decisions (Saterbak et al., 1999).

The objectives of this study were to: (1) evaluate the comparative toxicity response of invertebrates, plants and bacteria to combinations of oil and soil; (2) determine the effect of bioremediation on toxicity reduction; and (3) determine relationships of analytical measurements of hydrocarbon contamination and ecotoxicity.

2. Methods and materials

Test methods and materials are summarized below and are described in detail (Dorn et al., 1998; Salanitro et al., 1997).

2.1. Test oils

Three oils tested in bioremediation studies were obtained from US locations, represent three different API gravity weights and compositions, and were the same as those tested in earlier studies in our laboratory (Dorn et al., 1998; Salanitro et al., 1997). The light crude oil, obtained from the Gulf of Mexico is characterized by high saturated hydrocarbon content and low aromatic content with a weight of 53 API gravity. The medium weight oil is characterized as middle range in saturates and high in aromatics with a 30 weight API gravity, and the third oil is represented as a heavy Southern California crude oil with low saturate content and 16–18 API gravity (Table 1).

2.2. Bioremediation tests

Detailed methods are described by Salanitro et al. (1997). Approximately 4.5 kg (5% w/w) of each of the heavy, medium, light oil was added to 95 kg wet wt Norwood (∼75 kg dry wt) or Norwood/Baccto (∼70 kg dry wt) soil and sieved (⩽2 mm) (Table 2). The sieved oily soil (∼5% w/w) was mixed on plastic sheeting to allow aeration and 2–3 d artificial weathering. A significant fraction of volatile hydrocarbon fraction was lost (∼40–95% BTEX was volatilized during mixing and weathering).

Table 2
Physical–chemical characteristics of clean test soils

Parameter	Norwood	Norwood/Baccto[a]
pH	8.2	7.1
Texture (%)		
Sand	23.8	23.3
Silt	61.1	56.4
Clay	15.1	20.3
Moisture retention (%)[b]	18.5–27.5	25–28.5
Total organic carbon (%)[c]	0.3	4.65
Nutrients (mg/kg)		
Kjeldahl-N	517	2921
NH_4-N	4	ND[d]
NO_3-N	16	469
PO_4-P	59	145
Total-P	315	473

[a] Mixture of 75% (wet wt) Norwood silty loam and 25% (wet wt) Baccto® topsoil (sandy loam; 22.7% total organic carbon); [b] g water/100 g soil at 1/3 bar; [c] Walkley–Black method; [d] Not determined.

Fertilizer solution was added to each oily soil as N (100 g NH_4NO_3), P (40 g of K_2HPO_4) at a ratio of C:N:P = 100:1:0.2. Deionized water was added to 50–80% of the field moisture capacity. The fertilizer amended oiled soils (30 cm depth) were placed into 128-L stainless chambers (45 cm × 45 cm × 30 cm) and covered with Plexiglas. The chambers were swept continuously with 250 L/h humidified air. The water content was controlled during the incubation period to approximately 18–27% in the Norwood and 25–28% in the Norwood/Baccto soils.

2.3. Earthworm toxicity test

Eisenia fetida (Allobophora) purchased from Carolina Biological Supply Company (Burlington, NC) served as the starter source of the continuous in-house culture for testing. Worms were reared in neutralized sieved (5 mm mesh) peat having 80% water weight in pre-rinsed polyethylene trays. The trays, each containing approximately 400 worms, were covered with thin polyethylene wrap. A controlled temperature (22 ± 2°C) room with continuous daylight fluorescent lighting (400–800 lx) was used for rearing and testing earthworms. *Eisenia* were fed moistened pelletized alfalfa (Natural Brand Alfalfa tablets) weekly which was spread thinly and evenly on the surface of the soil.

The test design utilized a minimum of five replicates of ten earthworms each and five concentrations of oil-dosed soil before and after bioremediation. Adult worms weighing 300–600 mg each with clitella were used in all tests. Water evaporation and animal escape were minimized by placing a loose lid over the test containers. Worm survival was observed after 7 and 14 days (USEPA, 1989). Unoiled Norwood or Norwood/Baccto soil was used for the dilution series in all experiments, corresponding to the oiled soil.

2.4. Microtox® Solid-Phase toxicity test

The Microbics Microtox Model M500 Analyzer and Microtox Solid-Phase test kit were used to measure oily soil toxicity to the luminescent bacteria, *Photobacterium phosphoreum*. After 20-min incubation in the solid-phase cartridge, the light levels were measured in the analyzer and reported as EC50 concentration for light decrease (Microbics Corporation, 1992). The test allows that soil pellet to contact the bacteria in solution and is corrected against a control for any light sorption. This provides exposure of soluble and insoluble sorbed contaminants to contact bacteria.

2.5. Plant germination and growth tests

The effects of oily soil on seed germination and early plant growth were measured for corn (*Zea mays*), wheat (*Triticum aestivum*), and wild oat (*Avena sativa*) seeds. One hundred seeds of each species per treatment were tested in control soil, freshly oiled soil and bioremediated soil (between 8 and 10 months). Seeds were checked for germination and plant growth (measured as average dry weight/plant) after 21 days germination (USEPA, 1989; OECD, 1984).

2.6. Data analyses

LC50 concentrations were calculated for earthworm survival using probit and binomial methods. Microtox EC50 concentrations for light inhibition were obtained from instrument output.

Multifactor analysis of variance (ANOVA) was used to evaluate soil and oil effects and possible interactions on earthworm survival and Microtox and soil, oil, and plant species effects on germination and growth. Worm LC50s, Microtox EC50s, and plant germination and growth NOECs were used. All analyses were performed at an $\alpha = 0.05$ using TOXSTAT® and STATGRAPHICS® software.

3. Results

3.1. Earthworms

Bioremediation of hydrocarbon contaminated soils significantly reduced toxicity to earthworms. Earthworms were most responsive to effects of oil as mitigated by time, soil type and oil type. The results of all of the earthworm tests using all three oils in each of the two soils before and during bioremediation are shown in Table 3. The light oil was the most toxic oil in either of the two soils and was remediated with toxicity reduction increasing through the period of remediation. Heavy oils were least toxic showing LC50s of 100% or greater after 2–3 months time in either soil. These data show that toxicity reduction is achieved within several months under all conditions. There appears to be an increase in soil toxicity from the initial spiking period (month 0) and one month for some of the soils. This has been observed in several other tests conducted in our laboratory. Whether this is an artifact of heterogeneity of the soils or a phenomenon related to degradation of the oily soils is unclear. Fig. 1 compares those three factors representing the mean and ±1 SD for the entire Table 3 combined data. There were significant differences in all toxicity data between Norwood and Norwood/Baccto soils (Fig. 1A). Significant differences were observed with lower toxicity in the high organic carbon Norwood/Baccto soil with toxicity ranging between LC50 of 1–100% soil spiked with oil at test onset and diminished to LC50 > 100% by day 90 in all three oiled soils. Norwood soil with each of the three oils was

Table 3
Toxicity of different oils in two soils to earthworms before and during bioremediation. Values are expressed as earthworm LC50 in percent soil

Months	Norwood			Norwood/Baccto		
	Heavy	Medium	Light	Heavy	Medium	Light
0	22	4	1	100	10	1
0.5	26	9	1	27	4	9
1	28	4	1	100	79	23
3	100	22	6	100	100	100
5	92	90	30	100	100	100
8	100	100	–	100	100	100
10	100	100	66	–	100	–
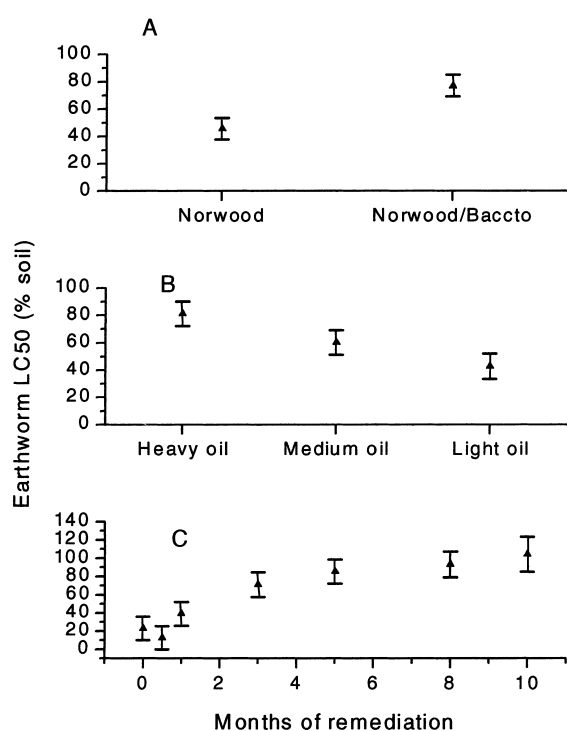
Fig. 1. Response of earthworms to three oils on two soils before and during remediation.

more toxic with LC50's ranging between 1–22% oiled soil at time 0 and remained toxic for at least 90 days and lasting for 360 d.

The light oil in the soils was significantly more toxic than medium and heavy oils. With mean LC50s of approximately 45, 60 and 80% oiled soil, respectively (Fig. 1B). A temporal plot of the LC50s with all soils and oils combined showed a steady decrease in toxicity from time 0 to 10 months (Fig. 1C). One hundred percent survival in all bioremediated soils was observed between 4–12 months (Table 4).

Significant effects of time on earthworm toxicity were observed with largest changes occurring after 90 days remediation treatment. After 90 days Norwood/Baccto soil was not acutely toxic (where the LC50 was ≥ 100% oiled soil) and after 240 days acute toxicity was absent in Norwood medium and heavy oiled soils, and lasted until toxicity was removed at 360 d in the light oil contaminated Norwood soil (Table 4).

3.2. Microtox

Toxicity was greatest for light oils in both soils and decreased with bioremediation (Fig. 2). Similar results were observed to those for earthworms, but Microtox sensitivity was less than for earthworms. All soils showed EC50 ≥ 100% after < 8 months remediation.

Table 4
Percent survival of earthworms in 100% soil during bioremediation

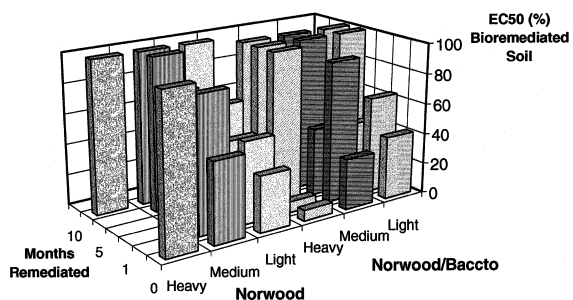| Months	Norwood			Norwood/Baccto		
	Heavy	Medium	Light	Heavy	Medium	Light
0	0	0	0	84	0	0
1	0	0	0	92	0	0
3	64	0	0	76	96	100
5	46	22	0	100	100	100
8	100	100	0	100	100	100
10	100	100	72	100	100	100
12			100			

Fig. 2. Responses of Microtox solid phase assay to combinations of soil, oil type and time of remediation.

3.3. Plants

The test plant species in the seed germination tests were generally equally sensitive to oil and were most responsive to the light oil in both soils (Fig. 3). Plant seed germination increased with oily soil remediation for light oils and was relatively unaffected in other soil/oil/bioremediation conditions. Germination was not significantly affected with heavy oil in either soils and was not affected by bioremediation. However, germination was reduced in soils containing light oil and increased with remediation. Relative plant growth for each species was similar in both control soils (data not shown) without oil but was lower in both soils with light and medium oils. Plant growth was a sensitive measurement but showed more variation than seed germination and was similar to that observed in our earlier screening experiments (Dorn et al., 1998).

Bioremediated Norwood/Baccto soil showed increased growth for light and medium oils compared to the freshly oiled soil. The response to remediation of the medium and heavy oils is consistent where the aromatic content is considerably lower than that found in the light oil.
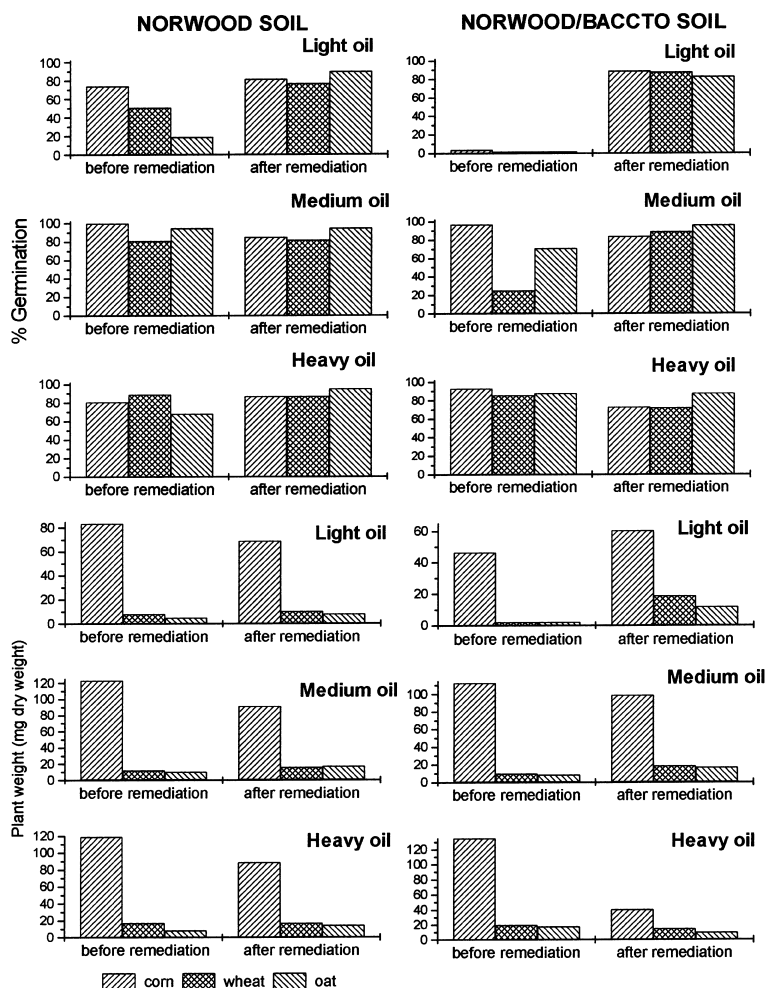

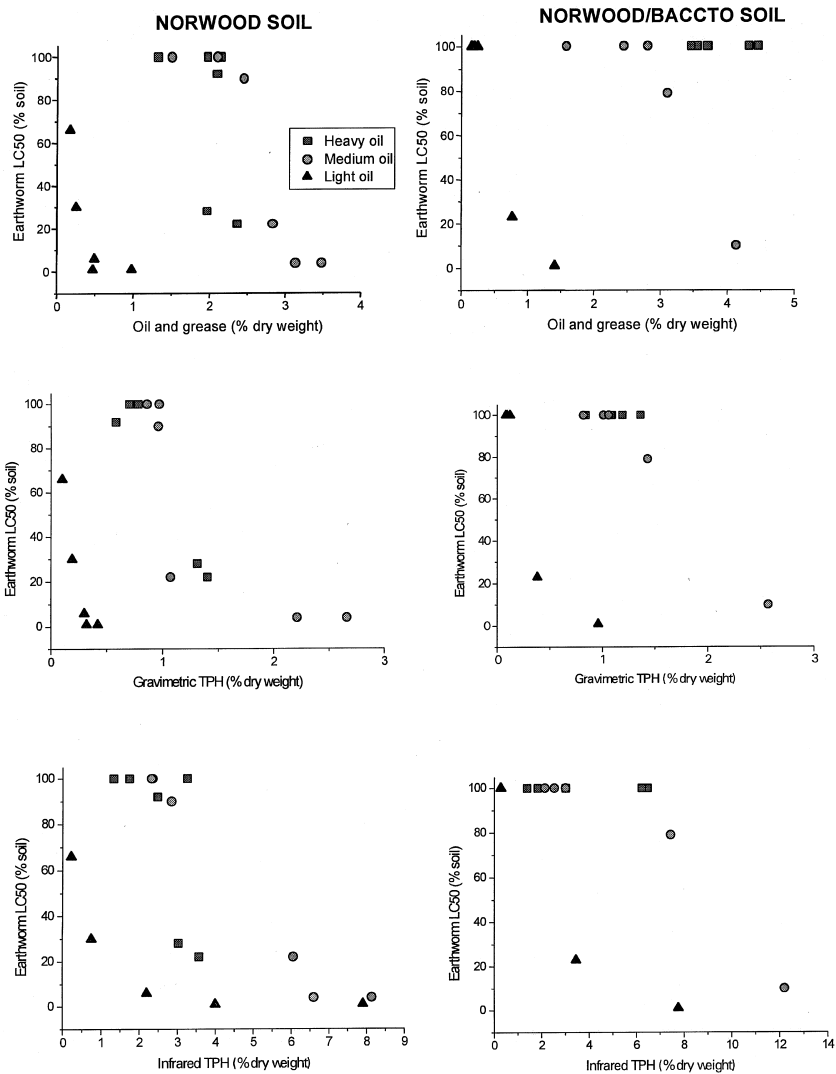Fig. 3. Responses of plant germination and growth to different oils and soils before and after remediation.

Fig. 4. Concentration-responses of earthworms to hydrocarbons as indicated by three analytical methods for the two soils. Data is compiled from all soils at all periods before and during remediation.

3.4. Comparison of analytical measurements for hydro-carbons

Initial concentrations of hydrocarbons in the two soils ranged from 35 700 to 122 200 mg/kg measured by TPH-IR. Concentrations in remediated soils after 10 months were below approximately 20 000 mg/kg in all soils for all three hydrocarbon measurements (TPH-IR, TPH-gravimetric, and gravimetric – oil and grease) (Salanitro et al., 1997). Rates of loss of the hydrocarbons from the soils were on the average highest in the Norwood/Baccto soil ranging from 13 to 81%/month as measured by TPH-gravimetric analysis. Hydrocarbon loss in Norwood soils ranged from 23 to 31%/month for the three oils.

A comparison of the concentration response of earthworms to oils at all time periods in the two soils as measured by gravimetric oil and grease, total petroleum hydrocarbons by infrared and also by gravimetric analysis showed no significant correlations (Fig. 4). The figure(s) show all of the equivalent hydrocarbon measurements at soil LC50 concentrations. There is a strong concentration-response relationship but no one analytical method provided a more precise measurement. However, there is a trend that indicates toxicity may correlate with hydrocarbon concentration and is best with measurements using TPH analysis. This conclusion was also affirmed in follow-up studies using field soils with hydrocarbon contamination (Saterbak et al., 1999).

A comparison of the three hydrocarbon measurements (TPH-IR, TPH-gravimetric, and gravimetric – oil and grease) to earthworm toxicity for the Norwood soil with heavy oil show considerable decrease in hydrocarbon concentration on the soil. There was an initial decrease in toxicity from 22% to >90% LC50 of soil corresponding to the hydrocarbon decreases. From approximately 4 months onward, there is no acute toxicity (LC50 $\geqslant$ 100% soil), and the hydrocarbon concentration continues to decrease. After eight months remediation earthworm percent survival in 100% soil is 100%. Fig. 5 illustrates this relationship for one soil and oil condition (Norwood soil/heavy oil). With respect to gravimetric oil and grease, the LC50 for earthworm survival expressed in mg/kg, shows that the LC50 increases as remediation progresses. This would indicate that this analytical technique is not a direct indicator of toxicity, otherwise the LC50 concentration would be equivalent over time. The weathering process is proposed as playing a role in changing the hydrocarbon composition and affecting toxicity. In each of the soil/oil combinations the gravimetric oil and grease concentration where 100% earthworm survival is observed ranges between 110 mg/kg in light oiled soils to 3470 mg/kg in heavy oiled soils.

4. Discussion

These results confirm the effect of active bioremediation upon reducing the toxicity of oiled soils to bacteria, invertebrates and plants (to a lesser degree). Again, we confirm that the earthworm is a sensitive test surrogate for oily soil, and indicates that the 14-d lethality bioassay measurement provides a reasonable approximation of soil toxicity for hydrocarbons. In our previous work (Dorn et al., 1998), this study, and that using field soils (Saterbak et al., 1999), earthworms were most responsive with the 14-d test and indicative of soil toxicity.
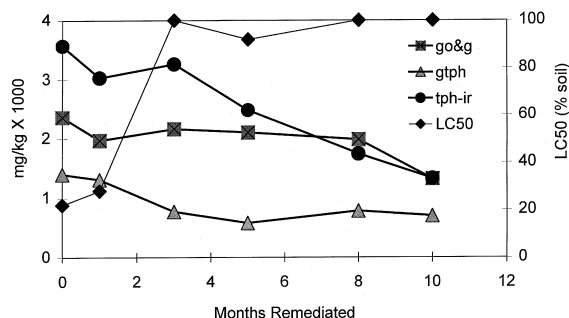

Fig. 5. Comparison of earthworm toxicity and analytical measurements for heavy oil in Norwood soil during bioremediation. Analytical measurements are gravimetric oil and grease (go&g), gravimetric TPH (gtph), and TPH by IR (tph-ir).

Similar approaches have been assessed for determining whether bioavailability can be modified by physical and chemical alternations of contaminated soils (Loehr and Webster, 1997). A recent study used soil washing to determine whether metal contamination could be removed from soil using ecotoxicity measurements and showed similar improvements from initial conditions (Chang et al., 1997).

Attempts to correlate toxicity with hydrocarbon measurements are difficult in contaminated soils. Contaminated soil quality can be categorized where: concentrations above a level are deemed toxic and require remediation and more careful assessment; below a certain level are considered non toxic and may be reduced in priority unless there are special considerations, and; those in the transition area that may require further assessment or be compared to other site specific data. Using this simple approach, our attempts to correlate toxicity with measurements of hydrocarbon by TPH-IR have shown that soils with hydrocarbon levels <4000 mg/kg have little effect on surrogate species while concentrations above 10 000 mg/kg show significant inhibitory effects for the soils and oils tested (Saterbak et al., 1999). They also observed concentration-response curves for earthworm 14-days acute survival as a function of TPH by GC suggesting that technique as an indicator of acute toxicity to *E. fetida*. The TPH by GC method measures C_6–C_{25} hydrocarbons which are the more volatile, soluble, and biodegradable constituents in crude and may provide an indicator of acute toxicity to worms.

Our study shows that light oils with high aromatic content are initially acutely toxic to surrogate soil species but lose this toxicity after active bioremediation. Evidence of low to no acute effects of heavy oil in high organic carbon soils indicate that these oily soils (with <5% oil w/w/) pose little impact to populations of soil dwelling organisms (e.g., earthworms). These results also demonstrate the active response of soil bioremediation can enhance recovery of soil and reduce toxicity to functional restoration of the soil for colonization of earthworms and plant reintroduction.

Species sensitivity is greatest for earthworms in all of the tests compared to the plant and Microtox assays. The highest response is observed in the Norwood soil with light oil. In that soil, the earthworm LC50 was 1% soil compared to 30% for Microtox and 30% oat seed germination. Similar response was observed in the Norwood/Baccto soil with light oil with all species responding. In other soils, plants were relatively insensitive to effects on germination.

Few studies have linked measured hydrocarbon concentrations to ecotoxicity data. Recent activities to approach an understanding of specific benchmark values for hydrocarbons resulting from residual oil have considered bioavailability and sequestration measurements

to predict toxicity. However, attempting to identify one specific hydrocarbon measurement for demonstrating an environmentally acceptable endpoint is in the future (Chang et al., 1997; Anderson et al., 1999) and subject to further study.

5. Conclusions

1. Bioremediation was effective in reducing oily soil toxicity to earthworms and Microtox decreasing the effects on germination and growth in plants.
2. Aromatic fractions in light oils may be responsible for the acute toxicity in soils.
3. No specific hydrocarbon measurement (TPH-IR, TPH-gravimetric, or gravimetric – oil and grease) was significantly correlated to toxicity in all oils.

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