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Full Length Research Paper

Comparison of moisture management methods for the bioremediation of hydrocarbon contaminated soil

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Different moisture management methods were compared for biodegradation efficiency in sandy and organic soils. The conventional method consisted in maintaining the soil moisture at approximately 50 to 75% field capacity accompanied by daily aeration and mixing. In the test method, the soil was allowed to dry out completely for three to four days after which the soil was moistened to 50 to 75% of field capacity and mixed daily for five days. In the test method the drying and moisturizing cycles were maintained throughout the experiment. There was no difference in treatments in the sandy soil, both treatments resulting in the detoxification to background levels within five weeks. During the processing of the organic soil, an increase in toxicity was observed, apparently due to increased availability of hydrocarbons, and possibly due to the production of toxic intermediates of biodegradation. The transformation rate in the test method was 22% less than in the conventional method, although this transformation started at least four weeks earlier than in the conventional method. Based on these observations, a combination of drying (to increase bioavailability) and conventional moisture management (to stimulate hydrocarbon degrading microorganisms) is recommended.

Key words: Remediation, toxicity, petroleum.

INTRODUCTION

In the south-eastern region of Mexico, petroleum industry operations are varied and constitute an important potential source of air, water and soil contamination (Adams et al. 1999). For this reason, priority programs have been established in the industry to remove or mitigate the environmental impacts caused by waste. In the case of soils affected by spills of crude oil or derivatives, restoration techniques have been implemented. Among these is bioremediation, which is optimal for low to medium molecular weight hydrocarbons (Adams et al., 1999; Atlas, 1986; Zeyer et al., 1986). In this process, the hydrocarbons in the soil are biodegraded by the application of nutrients and the increase in the oxygen content by mechanical methods (King et al., 1992; Yakubu, 2007; Zeyer et al., 1986). The levels of nutrients and control of physical factors (oxygen, moisture, temperature, etc.) affect microbial activity (Atlas, 1981, 1984; Dibble and Bartha, 1976; Gibbs et al., 1975). An example of this is the soil moisture, which is related to aeration due to the fact that the diffusion of oxygen in water is very low. In a soil with a high concentration of organic material, including hydrocarbons, the aerobic heterotrophic microorganisms quickly consume the oxygen present in the soil if it is not aerated sufficiently; thereby producing anaerobic microhabitats. However, the mineralization and humification reactions carried out by the oil degrading microorganisms are almost exclusively aerobic reactions, and the production of anaerobic microhabitats in the soil can severely limit the overall biodegradation rate. Usually, in bioremediation projects, an ideal of about 50 to 75% of the field capacity of the soil is maintained,

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Abbreviations: TPH, Total petroleum hydrocarbon; **CEC,** cation exchange capacity; **ESP,** exchangeable sodium percentage.

which is generally sufficient to maintain the soil moist enough for a good microbial activity, while at the same time low enough to allow for sufficient aeration (King et al., 1992).

None-the-less, some experience has been gained which suggests that other moisture management methods may be beneficial. Bartha and El-Din (1993), ran respiration tests to evaluate the biodegradability of petroleum hydrocarbons and other carbon and energy sources (n-hexadecane, benzoic acid, adipic acid and glucose) in soil. Preliminary observations in this study indicated that better biodegradation rates were obtained if the soil was completely dried at the beginning of the experiments. Apparently, the substrates are more evenly distributed in the soil by drying and are more available to micro-organisms and their enzymes.

Occasional drying as a moisture management method is also supported by field observations. During the remediation of hydrocarbon contaminated sites, some remediation companies have problems with the availability of equipment and personnel, and soils are not always moistened and mixed according to schedule. From these field experiences it appears that letting the soil dry out occasionally and subsequently restoring the moisture to 50 to 75% of field capacity, may not greatly affect the overall bioremediation rate. The observed rate is about the same (or maybe even a little better) than that expected if adequate soil moisture was always maintained (Personal communication, S.R.T. Severn, Pacific Technologies, Inc., Belleview, Washington, USA). As a matter of fact, scientific controls are not employed in these commercial projects, and this observation needs to be confirmed in controlled experiments.

To measure the effectiveness of the remediation, toxicity (besides just hydrocarbon concentration) can also be helpful as a criterion. Sometimes, toxicity may even be a more important criterion especially as it relates to the *impact* that hydrocarbons may have on soil organisms. For example, Overton et al. (1997) found that toxicity was proportional to low molecular weight hydrocarbons in marshy soils and sediments, but there was no correlation between the concentration of heavier hydrocarbons and toxicity. Adams et al. (1999) also cites work on the remediation of drilling cuttings in which the hydrocarbon concentration was reduced by only ~10% but the overall toxicity was reduced more than five times. Other authors have also proposed toxicity as the main criterion for soil remediation (Alexander, 1999), and recently the risk based remediation plan for a 22 Ha site in southern Veracruz, Mexico has been approved by state environmental authorities using background toxicity as the sole cleanup criterion (SEMARNAT, 2007).

The objective of the present study was to determine whether occasional drying during the remediation of hydrocarbon contaminated soil does indeed increase the rate of microbial mediated transformation using toxicity as the main remediation criterion.

MATERIALS AND METHODS

Site selection and soil sampling

For this study, it was considered important to work with real contaminated soils resulting from inadequate petroleum production or transport, typical of the oil pollution problems faced in the southeast Mexico region, rather than collecting soil and artificially contaminating it. In this study, the soil was not experimentally contaminated but rather collected from the field already contaminated. This makes it more difficult to control conditions, such as the level of contamination, for example, but the results are more likely to represent actual field conditions during a real remediation project.

Two sites containing contaminated soil with very different physical-chemical characteristics were selected, one with organic rich soil (a histic Gleysol) and one with sandy soil (an Arenosol). This selection was made due to: (1) The fact that these soil types are common in the petroleum producing region of southeastern Mexico; (2) they differ considerably with respect to field capacity and internal drainage and, (3) considering that they may respond differently to alternative moisture management methods.

The first site (organic rich soil) was located in the Ogarrio oil field at 18° 26' 08.12'' N and 93° 14' 27.11'' W (Figure 1). This is a marshy area consisting of a mosaic of marsh and floodable tropical forest. The marsh is dominated by cattails (*Thalia geniculata*) and giant flatsedge (*Cyperus giganteus*) and the floodable forest is dominated by Guiana chestnut (*Pachira aquatica*). The surface soil layer (epipedon) is predominately organic (histic). The soil in this area has been described as an association of eutric Gleysols and Histosols (Palma and Cisneros 1996) and has very high organic matter content (80%), a Cation Exchange Capacity (CEC) of 70 cmol (+)/Kg, a pH of 4,1, a salinity of 1,6 dS/m and an Exchangeable Sodium Percentage (ESP) of 5% (Zavala et al. 2005). In the selected installation (Ogarrio No. 1247), there was a small spill of crude oil about one year prior to sampling. The oil from this field is light, having an API gravity of 35, and is very low in residuals (9, 0%) and asphaltenes (0.24%, Table 1).

The second site is located in a coastal area at 18° 26' 08.12" N and 93° 14' 27, 11" W. The surrounding land is used for coconut plantations and it is very near to the coast (~200 m). The soil type is Arenosol, consisting of a loamy sand (80:10:10; Sand:Silt:Clay), with very low organic matter content (0.3%), low Cation Exchange Capacity (10 cmol (+) / Kg), a pH of 6,3 and low salinity, 1,6 dS/m and an ESP of 2% (Palma and Cisnersos, 1996). The soil in the site selected (Puerto Ceiba No. 101-A) was contaminated with very weathered and partially burned hydrocarbons from a burn pit. The oil from this field has an API gravity of 31, but it has more than five times the residuals than the oil from Ogarrio and 20 times the amount of asphaltenes (Table 1). This, as well as burning and environmental exposure in a tropical environment, made the hydrocarbons in this second site much more weathered and recalcitrant.

Soil collection was based on field physical observations indicating hydrocarbon contamination (color, consistency). Approximately 50 Kg of soil was collected from the Puerto Ceiba oil field and 70 Kg from the Ogarrio oil field, in both sites to a depth of 30 cm. Subsequently, the soil was deposited in black polyethylene bags and kept at ambient temperature.

Experimental design

This consisted of trying two moisture management methods in two different soils (2 x 2 block) using three replicates for each treatment (Table 2).

Preparation of bioremediation experimental units

Three replicates were prepared for each treatment for the organic

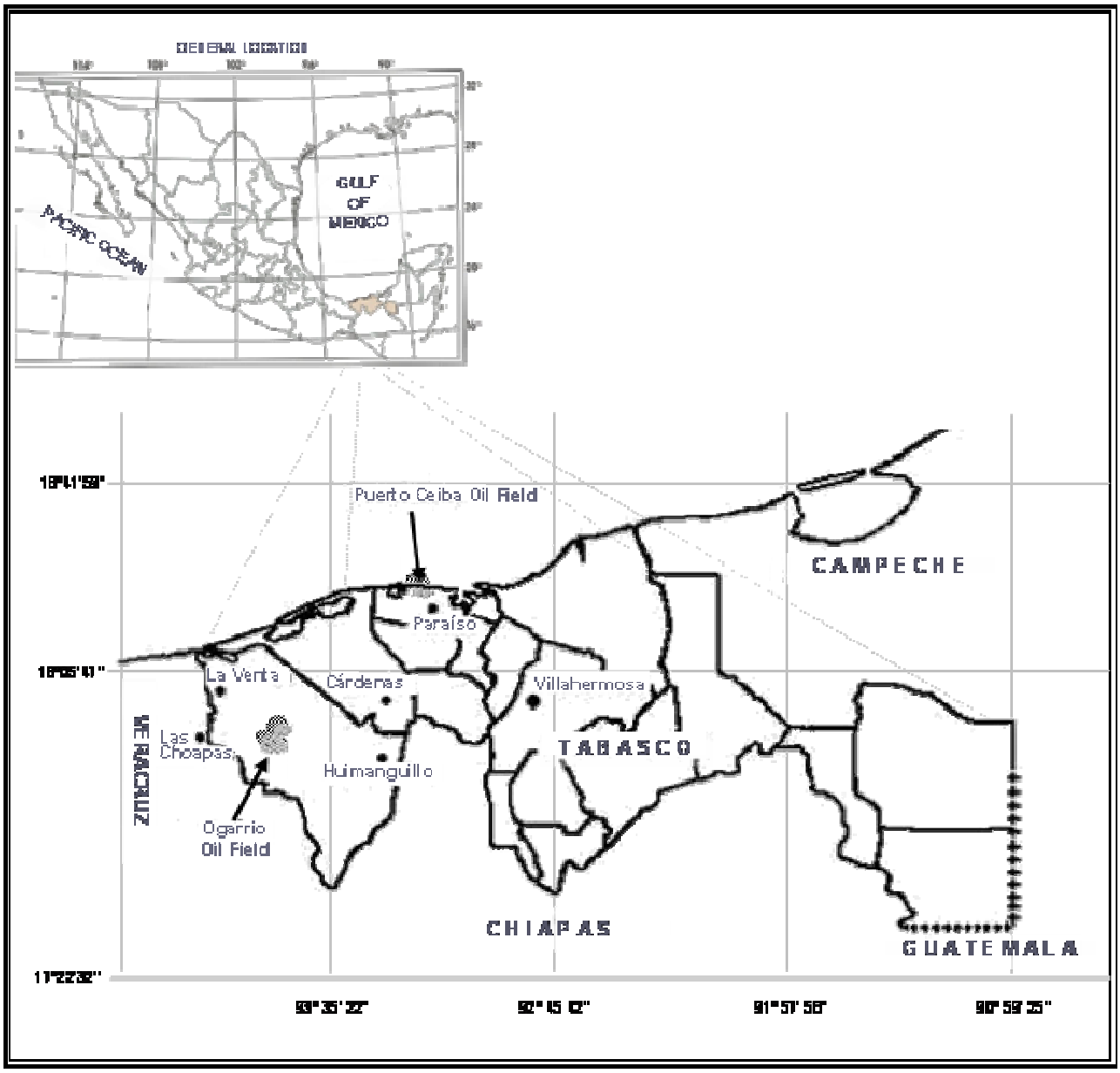

Figure 1. Location of soil sampling sites.

Table 1. Physical-chemical characteristics of crude petroleum from the Ogarrio and Puerto Ceiba Oil fields.

Characteristic	Ogarrio Oil Field	Puerto Ceiba Oil Field
Gasolines (%)	30	20
Kerosines (%)	9	12
Gasoils (%)	39	20
Total distilation (%)	90	52
Residuals (%)	9	47
API gravity (60^{O}F)	35.38	31.16
Asphaltenes (%)	0.24	4.77
Cinematic viscosity (37.8^{O} C)	5.46	6.91

Source: PEMEX Exploración y Producción. 1996, 1997.

Table 2. Experimental design in 2 x 2 block with three replicates.

Soil type	Moisture management method	
	Convencional method	Test method
Organic (Ogarrio oil field)	3 replicates	3 replicates
Sandy (Puerto Ceiba oil field)	3 replicates	3 replicates

Table 3. Mixtures of conditioners and contaminated soil.

Component	Organic soil (Ogarrio oil field)	Sandy soil (Puerto Ceiba oil field)
Contaminated soil (%)*	72.8	72.0
Partially composted cacao husk (%)	13.6	16.0
Sand (%)	136	____
Dark alluvial soil (%)	_____	12.0
TPH** (mg/Kg)	285 000	27 200

*The percentages were calculated on a volume basis of the total mixture. **Total petroleum hydrocarbons, (dry weight basis).

soil (Ogarrio oil field) and for the sandy soil (Puerto Ceiba oil field), making a total of 12 experimental units. The soil collected was already contaminated with hydrocarbons by petroleum industry operations in the field. Soil conditioners were added to improve water retention, internal drainage and aeration, and nutrition. The amount of soil conditioners to add was estimated from preliminary test that were ran on each soil. Subsequently, the mixtures of contaminated soil and conditioners were prepared according to Table 3. Note: The soil was not experimentally contaminated; these are concentrations in soil that was collected in the field (already contaminated) after the soil conditioners were added.

Volumetric equivalents of 7 L of the soil mixture (contaminated soil and conditioners) were placed in round plastic trays 38 cm in diameter and 14 cm deep. In each treatment unit an agricultural fertilizer (Grofol 20 – 30 - 10, Grupo Bioquímico Mexicano, S.A. de C.V.; Rodríguez 1997) was added along with horse manure to a final nitrogen concentration of 100 mg/Kg. The horse manure supplied ~5% of the total nitrogen added. These trays were incubated indoors at approx. 28 to 30°C.

Moisture management in experimental units

Traditional method

Moisture was maintained at approximately 50 to 75% of field capacity and the soil was mixed daily with a small garden shovel for twelve weeks. To determine appropriate moisture levels, the moistened soil was compared visually to sealed jars with the same type of soil previously wetted to 40, 50, 60, 70 and 80% of field capacity. The field capacity of the soil was predetermined by weight difference between completely dry soil and saturated soil which had been allowed to free-drain overnight, according to Zavala et al. (2005).

Test method

The soil was allowed to completely air dry for three or four days and subsequently moistened to 50 to 75% field capacity and mixed daily for five days. This cycle was repeated continuously for the twelve week treatment period.

Sampling for toxicity and hydrocarbon concentration

Each week a representative sample of 50 g of soil was collected from each experimental unit with a small garden shovel after thoroughly mixing the soil, collecting twelve samples each week for a total of twelve weeks.

Toxicity analysis

Acute toxicity was measured with a modified Microtox method (SECOFI 1995) considering the recommendations of the manufacturer (Microbics Corp. 1995). This test uses bioluminescent marine bacteria which produce less light in the presence of toxic substances. The decrease in bioluminescence is measured in an analyzer which is similar to a spectrophotometer, except that the bacteria are the light source (no extra light source is used). This test is useful to determine if the concentration of hydrocarbons in the soil are truly toxic, considering that this property may vary considerably according to bioavailability in the soil. Two grams of soil were mixed into 100 ml of deionized water, mixing until a dark color was observed in the solution. Subsequently, the soil extract was filtered with a Whatman no. 40 filter and the filtrate was analyzed in a Microbics 500 analyzer in four serial dilutions according to the manufacturer's manual. Each treatment method was tested in triplicate each week.

Determination of total petroleum hydrocarbons

In this study, toxicity was used as the primary criterion for soil remediation and sampling was made weekly and in triplicate for each treatment for this parameter. However, so as not to change the proportions of soil in the treatment trays too much during the testing (and thereby inadvertently cause unplanned for changes in the biodegradation rate), it was necessary to limit the amount of sample collected for testing. Therefore these samples were prioritized for toxicity analyses and were not always available for hydrocarbon analyses. To obtain a more representative sample and reduce variability, small amounts (about 20 g) of soil from each of the three experimental units per treatment were collected periodically and combined to make a composite sample for each

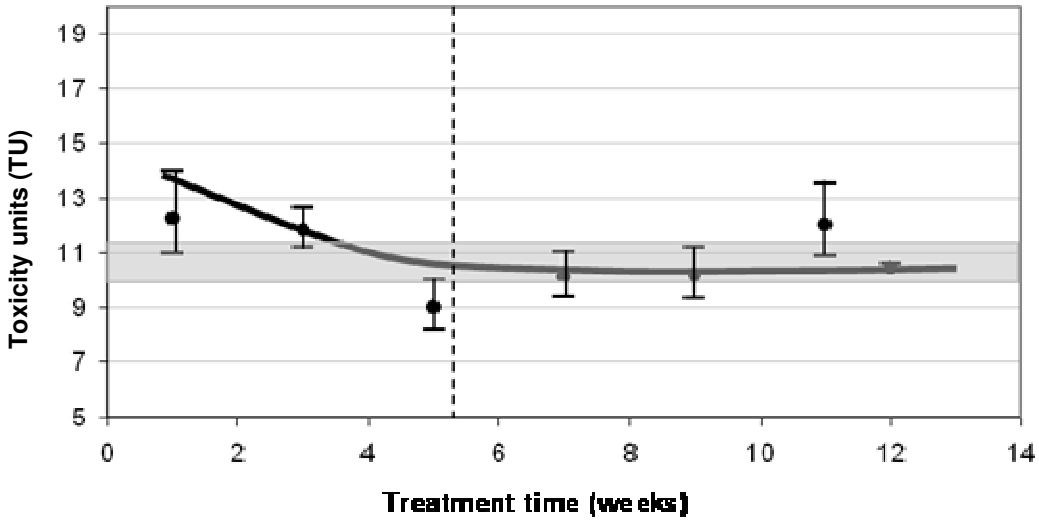

Figure 2. Acute toxicity in sandy soil – conventional method. Points represent averages and error bars represent one standard deviation. The gray shadow represents background level including one standard deviation.

treatment.

At the beginning, in the middle and at the end of the treatment period (12 weeks) single composite soil samples were analyzed by a modified EPA 9074 method (Dexsil Corp., 1998). The modification included calibration on a dry weight basis and multiplication by an extraction efficiency factor that compensates for the portion of the total hydrocarbons which are only poorly soluble in the extraction solvent (methanol). Additionally, at the begging and at the end of the treatment period, the TPH concentration was also determined gravimetrically in single composite samples according to McGill and Rowel, (1980) using dichloromethane as the extraction solvent. This strong solvent was selected by McGill and Rowel, (1980) due to availability and the ability to extract practically all hydrocarbons from the soil.

RESULTS AND DISCUSSION

Determination of background toxicity

In this analysis, the concentration of soil (in a water extract) which reduces the bioluminescence 50% was determined (half maximal effective concentration, EC_{50}). In this presentation a *lower* value of EC_{50} denotes a *higher* toxicity. To simplify this interpretation, EC_{50} values were converted to Toxicity Units (TU) in which a higher value denotes a higher toxicity according to the following formula (SECOFI 1995):

$$TU = 1/ EC_{50}$$

where EC_{50} is expressed as a fraction (example, 100 000 ppm = 0.1).

Before evaluation of the remediation effectiveness in the treatments it was necessary to be able to compare the toxicity to background soil toxicity. Background toxicity was determined using the same method (Microtox) on uncontaminated surface soil of the same type collected near (< 50 m) the contaminated sites. In the organic and sandy soils the background toxicities were 11.20 ± 1.07 TU and 10.52 ± 1.07 TU, respectively. These levels represent the natural, low level toxicity that these soils show when using this bioassay, due to naturally occurring toxins in the soil.

Comparison of moisture management methods in sandy soil

In the sandy soil, the two different moisture management methods had very similar results. At the begging of treatment, the toxicity in the soil under conventional and test moisture management methods had toxicities of 12.00 TU and 14.73 TU, respectively. After five weeks of treatment, the toxicity was reduced to background levels (10.52 TU) in both treatments (Figures 2 and 3). It is probable that achieving this reduction to background level in so short a time was due to the relatively low TPH concentration and to the weathered nature of the hydrocarbons. It is generally known that very weathered hydrocarbons are low in overall toxicity (Adams et al., 2006; Adams and Morales-García, 2008; Edwards et al., 1997; Overton et al., 1997). Furthermore, the sandy texture of this soil probably allowed for a good aeration and favors high rates of hydrocarbon biodegradation (Adams et al., 2002; Odokuma and Dickson, 2003). Although some sandy soils may have poor microbial activity (Adams et al., 2002) due to the very low quantities of nutrients in soil with low concentrations of organic material and clays, this loamy sand (which had 10% clay, 10% silt, and 0.3% organic material), appears to have

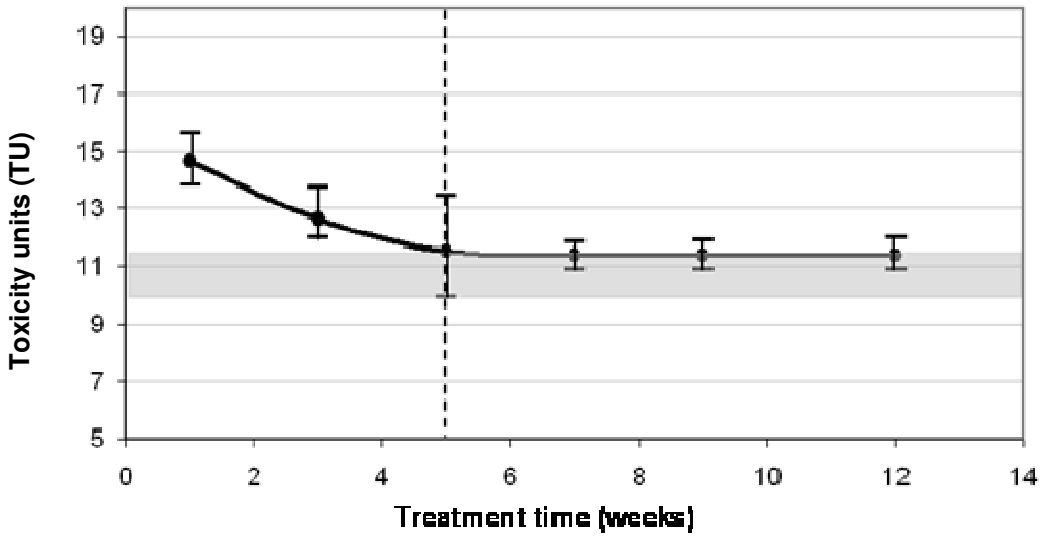

Figure 3. Acute toxicity in sandy soil – test method.

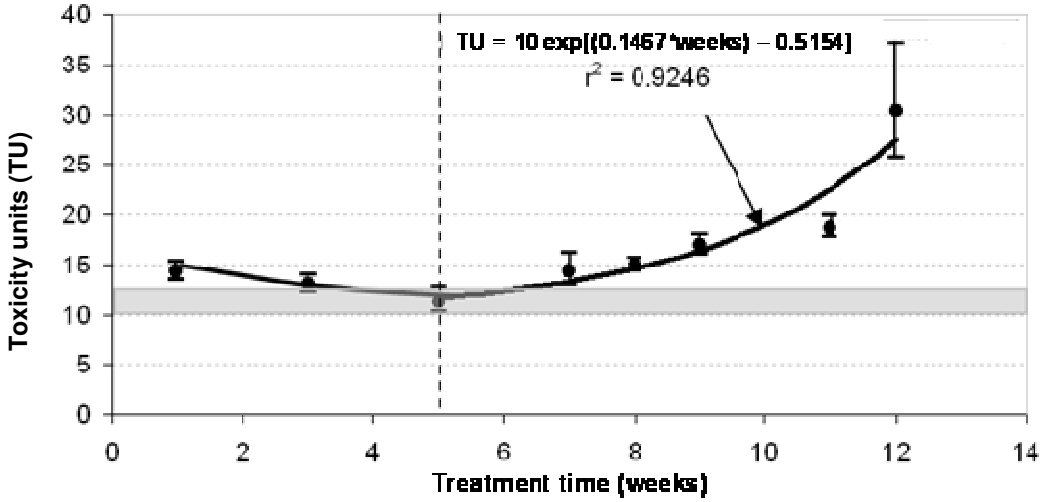

Figure 4. Acute toxicity in organic soil – conventional method. Points represent averages and error bars represent one standard deviation. The gray shadow represents background level including one standard deviation.

been sufficiently fertile (at least after the addition of the soil conditioners, fertilizer and horse manure) to biodegrade the remaining toxic hydrocarbons relatively quickly.

Comparison of moisture management methods in organic soil

In the organic soil with the conventional moisture management method, a preliminary reduction of acute toxicity to background levels was observed, similar to that found in sandy soil (Figure 4). This may be due to the biodegradation of the more available hydrocarbons in the soil. None-the-less, starting at five weeks the toxicity began to increase. It is possible that this was due to the liberation of hydrocarbons trapped in the organic material. With the advance in treatment, the microbial decomposition of organic fibers could have produced this result. It is also possible that during their biodegradation, certain hydrocarbons where transformed to more toxic metabolic intermediates such as alcohols or aldehydes (Brock et al. 1994; Manahan 1992; Rochkind et al. 1986). This process continued during the following seven weeks (Figure 4). Observations made on the consistency of the soil are congruent with the hypothesis that the hydrocarbons were trapped in organic tissues and latter released. At the begging of treatment the soil consisted principally of

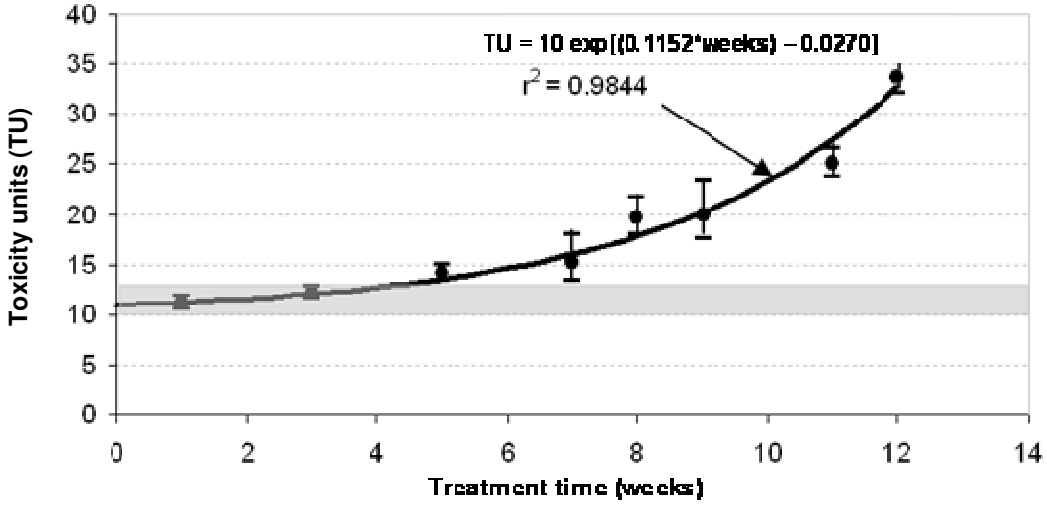

Figure 5. Acute toxicity in organic soil – test method.

only slightly decomposed large stalks, roots and other vegetable tissues, but after four weeks these started to break up into smaller fragments. This also allowed for a better and more homogenous moistening of the soil. Previous to this transformation, the presence of high concentrations of hydrocarbons in the soil made moisture management difficult due to the formation of a semi-impermeable layer that greatly reduced water infiltration.

In contrast to the soil with conventional treatment, the organic soil under the test treatment showed an increase in toxicity almost immediately (Figure 5). After the first week of treatment, the toxicity began to increase. It is possible that this test method is more effective in initiating the release of hydrocarbons trapped in the organic material by a physical method, such as the expansion and contraction of the vegetable tissues during wetting and drying. It is probable that during the following weeks the hydrocarbon availability (and therefore the toxicity) increased by these physical methods as well as the microbial decomposition of the organic material, as observed in the conventional test method.

Although this process (which results in the increase in toxicity) started earlier in the test method, the transformation rate was slower. To compare these rates, logarithmic regression analyses were preformed on the toxicity functions considering background toxicity as a base (figures 4 and 5). The correlation coefficients for both treatments were very good (r^2 = 0.925 for the conventional method and 0.984 for the test method). This suggests that these transformations (or liberation of toxic hydrocarbons) were of biological origin, probably related to the growth of the microorganisms involved. The logarithmic slope of this function in the conventional treatment was considerably greater (22% more) than in the test method. This implies that, although the inferred liberation of toxic hydrocarbons occurred four weeks earlier in the test method, that once it started in the conventional method, the rate was much greater. It is very probable that in the test method the intervening dry periods reduced the overall transformation rate by adversely affecting microbial activity, reducing the rate of hydrocarbon liberation and/or the production of more toxic metabolic intermediates.

Evaluation of total petroleum hydrocarbon (TPH) reduction

The reduction in the TPH concentration is presented in figures 6 to 9. As shown in figures 6 and 7, the reduction in the TPH concentration in sandy soil was very similar in the two treatment methods, resulting in a reduction of about 56% in the conventional method and 60% in the test method. It can also be observed that the TPH determination using methanol (EPA 9074) and dichloromethane (gravimetric method) are very similar, especially at the end of the treatment period.

With respect to the analyses in the organic soil a much greater difference was observed between the two treatment methods (Figures 8 and 9). In the conventional treatment an overall TPH decomposition of 59% was observed, while in the test method a TPH decomposition of only 38% was measured. With these single samples, and without the ability to run statistics on the data, it is not certain if this difference is real or due to random variation. However, the samples used were composite samples and thus much of the variability was reduced. Considering the magnitude of difference between the biodegradation percent in the conventional method vs. the test method, it is probably real. It may be due to the inactivity of hydrocarbon degrading microorganisms during the drying periods experienced in the test method. In both methods the experimental units were incubated uncovered indoors at approx. 28 to 30°C, but in the test

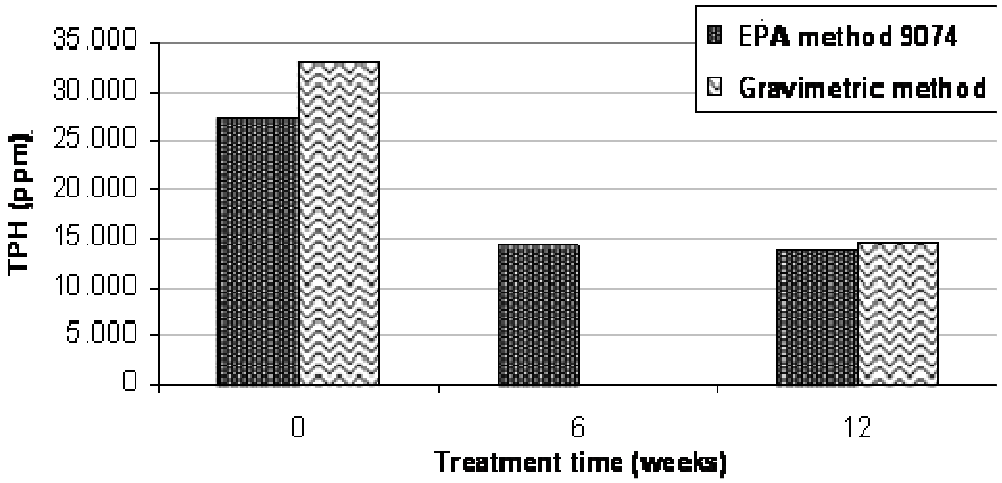

Figure 6.Variation in TPH Concentration in Sandy Soil - Conventional Method. Dark bars represent turbidimetric analyses run using methanol as the extraction solvent; grey bars represent gravimetric analyses using dichloromethane as the extraction solvent.

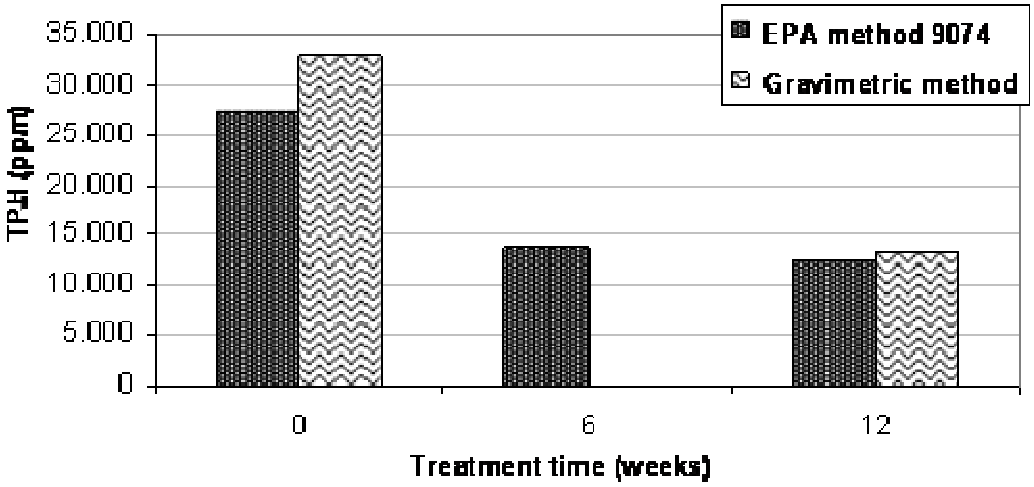

Figure 7. Variation in TPH Concentration in Sandy Soil - Test Method.

method the experimental units were allowed to dry to very low levels (< 40% Field Capacity), which is not sufficient to maintain good bacterial activity in soil (King et al., 1992; Paul and Clark, 1989).

One can also observe that the methanol extractable hydrocarbons in both treatments increased between the sixth and twelfth week of treatment using both moisture management methods in the organic soil. In the conventional treatment this increase was only about 21%, and may just be due to random variation, but in the test method it was 113% and is probably real. In these single samples, it cannot be determined with certainty if this difference is genuine, without statistical tests. However, considering that the analysis were run on composite samples and the magnitude of difference, especially in the samples from the test method, this difference is very likely to be real. It is probable that the *apparent* increase in hydrocarbon concentration is actually due to the decomposition of the organic fibers and liberation of the hydrocarbons, thereby increasing availability and extractability, especially with a more polar solvent (methanol). It is important to point out that this increase in availability was much greater in the test method than in the conventional method, and may result from greater physical fragmentation of the organic fibers in the soil during the wetting and drying cycles. At the end of this experimental treatment the TPH concentrations (as well as toxicity) were still very high and this material required a much longer treatment time to biodegrade the hydrocarbons to acceptable levels.

It is uncommon to observe these levels of biodegradation in material that is so contaminated. Often the

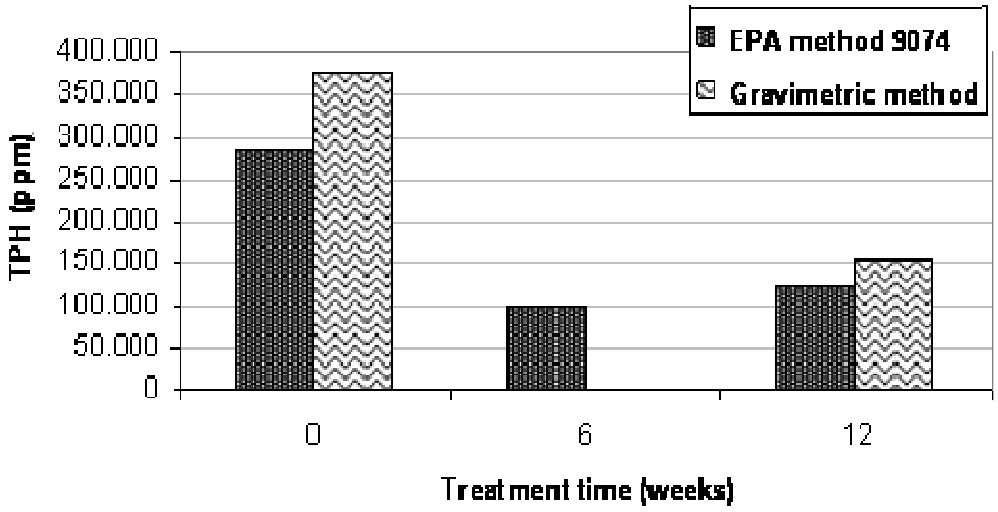

Figure 8. Variation in TPH Concentration in Organic Soil - Conventional Method.

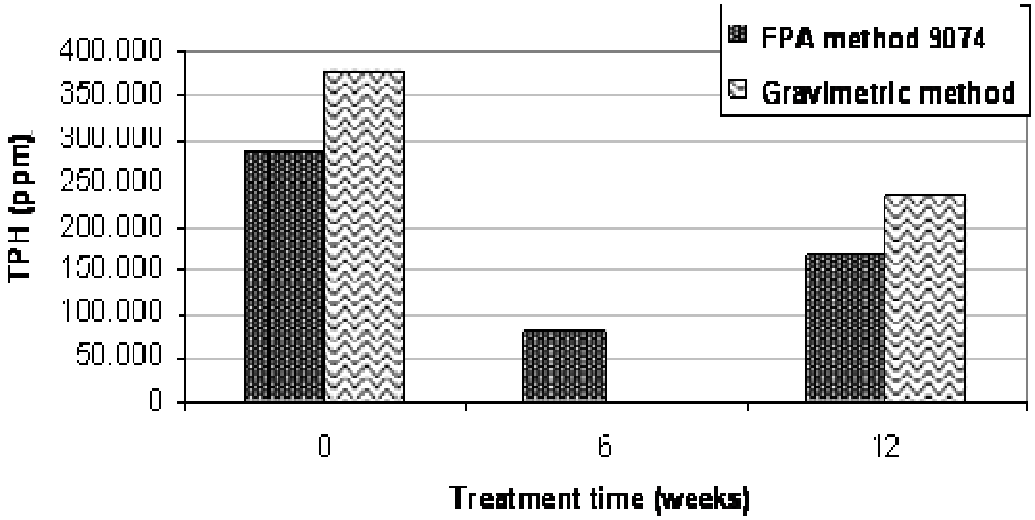

Figure 9. Variation in TPH Concentration in Organic Soil - Test Method.

very toxicity of the hydrocarbons, or alternations caused by the hydrocarbons in physical and chemical factors which affect soil fertility, impede the biodegradation at concentrations above about five to ten percent (Overton et al., 1997; Yakubu, 2007; Adams et al., 2002; Adams et al., 2008). None-the-less, there are antecedents of soils with very high concentrations of hydrocarbons being treated successfully by biological means, especially if the physical-chemical limitations are overcome, by incorporating soil conditioners, or if the soil naturally has high organic matter content. One example of this was the remediation of marshy soil contaminated with high concentrations (11%) of very weathered hydrocarbons (Adams et al., 2005). In that study the contaminated soil had concentrations of organic matter ranging from 21 to 56%, and an additional 4% of organic soil amendment was added. It appears that in these situations, the high organic matter content may be the key. It is known that in addition to improving soil fertility, soil organic matter also reduces bioavailability of organic pollutants in the soil and therefore, toxicity, even of very toxic compounds (Alexander, 1999).

This is probably the case in the present study. The petroleum from the Ogarrio oil field is light, with 35 ºAPI and only 9% residuals. This kind of petroleum is typically relatively toxic, but in this soil (histic epipedon, with 80% organic matter) the toxicity appeared to be mitigated by absorption into the organic fibers. It looks as if upon decomposition of these fibers, toxicity increased. However, the kinds of hydrocarbons present in this kind of

petroleum are also among the most easily degraded, being rich in medium weight aliphatics (Atlas, 1984; Potter and Simmons, 1998). Similar kinds of hydrocarbon mixtures have shown to be easily and rapidly biodegraded, especially in a tropical environment. For example, Odokuma and Dickson (2003) found overall biodegradation rates of Bonny Light crude oil (33 ºAPI) in a tropical soil to be nearly 90% with biodegradation half-lives of only 21 to 32 days. Similarly, Salazar (2002) reporting on the bioremediation of diesel based drilling cuttings, found half lives of 33 to 38 days in southeast Mexico (humid tropics). In passive, natural attenuation cases, these kinds of petroleum mixtures also degrade rapidly. For example, Díaz-Ramírez et al. (2009) reports on the passive biodegradation of gasoil following a catastrophic spill in southeast Mexico, finding a overall degradation rate of 58% in 17 weeks, corresponding to a half-life of 58 days, even with starting a concentration above five percent.

In the present study the overall biodegradation rate was 59% in 12 weeks, corresponding to a half live of 64 days. This is low for an active bioremediation project in the tropics, but comparable to natural attenuation of similar petroleum mixtures. It is consistent with what might be expected considering both the very high concentration of hydrocarbons, but also the relatively good aeration provided by daily mechanical mixing of this high organic matter soil.

Conclusion

Some of the results presented in this paper were not expected, especially the increase in toxicity observed in the organic soil (Ogarrio oil field). There are various explanations for this phenomenon and there are observations from other investigation which report similar findings. One example of this was found during the treatment of soil contaminated with heavy range hydrocarbons from a previous coal gas plant, where a reduction during the initial phase of treatment resulted in an increase in toxicity (Pradhan et al., 1997). In other research in which toxicity was measured using the Microtox bioassay in hydrocarbon contaminated wetlands in Louisiana (USA), is was observed that toxicity was correlated to the concentration of the most toxic fraction of hydrocarbon (light range) but not to the overall hydrocarbon concentration (Overton et al., 1997). Mid-range and heavy-range hydrocarbons typically present only very low toxicity (Adams et al., 2006; Edwards et al., 1997). It is possible, however, that intermediates of the hydrocarbon biodegradation pathways are more toxic than the original hydrocarbons, corresponding to an increase in toxicity during biodegradation and a corresponding decrease in the overall hydrocarbon concentration. For example, in the biodegradation of alkanes, the terminal carbon in the carbon chain is transformed in a series of biochemical reactions to alcohols, aldehydes, fatty acids, acetic acid and finally carbon dioxide. Some of these intermediates, especially the alcohols and the aldehydes are more toxic and reactive than the original hydro-carbons (Manahan, 1992). Likewise, many unsaturated aliphatics as well as aromatic compounds undergo an epoxidation reaction or are converted to oxides upon decomposition, which are more toxic than the original substrates (Brock et al. 1994; Manahan 1992).

In addition to the changes in toxicity per se, it is possible that an apparent increase in toxicity observed is due to physical changes in the bioremediated material. It is probable that the decomposition of vegetable organic matter, concurrent with the biodegradation of hydrocarbons, results in the liberation of the hydrocarbons which were adsorbed or absorbed by the organic matter. This results in a greater hydrocarbon bioavailability and as a result, greater toxicity. These kinds of physical changes were observed with the advance of the research project, especially after the fourth week of treatment. The results of the analysis for hydrocarbons using methanol as a solvent are congruent with this hypothesis.

Based on these findings one can conclude that in sandy soils with a relatively low hydrocarbon concentration (<5%) there is basically no difference between using the conventional method or the test method (with wetting and drying cycles). Thus there is no reason to implement a new treatment method for bioremediation under these conditions. Also, it shows that for these conditions at least, occasionally missing the moisture management schedule will probably not affect the overall rate of bioremediation.

However, for conditions in which the hydrocarbons may be partially sequestered in the soil organic material, a modified moisture management method may increase the bioavailability of the hydrocarbons and ultimately the biodegradation rate. In the present study evidence of early increased bioavailability was found in the treatment that began with a thorough drying of the soil, manifesting at least one month earlier. However, it appears that the overall rate of biological activity, as measured by changes in hydrocarbon bioavailability (toxicity), or reduction in the hydrocarbon concentration, was reduced, probably due to the drying itself. If this is true, a moisture management method including an initial thorough drying (to increase bioavailability) followed by normal maintenance at 50 to 75% of field capacity (to sustain a high microbial activity) would be recommended. Future studies with organic rich, contaminated soil with more comparable hydrocarbon concentrations (<5%) and with replicate analysis of hydrocarbon concentration would be useful for confirmation of these findings.

In organic rich soils, there may be in intermediate phase in the biodegradation in which the toxicity of the soil increases, either due to the production of toxic intermediates, or increased availability of the hydrocarbons. For this reason, it is important to completely finish comer-

cial bioremediation projects and include a bioassay (toxicity test) as a clean up criteria, using background levels as a reference.

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