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# Degradation of oil sludge by landfarming – a case-study at the Ghent harbour

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## Abstract

Large-scale landfarming experiments have been performed on a loamy sand soil. An amount of 1,350 $m^3$/ha oil sludge together with nutrients (N,P,K) and a bacterial inoculum were appplied at two different times over a five-year period. At both test periods, biodegradation of the hydrocarbons (HC) was best fitted with first order reaction kinetics with degradation rates ranging from about 4 g HC/kg dry soil per year to about 15 g HC/kg dry soil per year. Toxicity tests on the aqueous soil extracts as well as plant growth and worm tests on the landfarm soil showed no striking negative effects of residual hydrocarbons. Migration of oil, nitrate and phosphate to the groundwater was minimal. In view of the diversity of solvents recommended in the literature, twenty extractants were tested for their capacity to remove HC from the loamy sand soil. Chlorinated solvents, such as dichloromethane and chloroform, were the most effective. Yet, in view of its effectiveness and low toxicity, acetone appears a suitable solvent for the extraction of soils and sediments polluted with hydrocarbons. This case-study revealed that oil sludge can effectively be treated by landfarming, if appropriate technical measures are taken and a sufficient time (minimum 15 years) for bioremediation is provided.

## 1. Introduction

The concept of landfarming was developed and firstly practiced by the US oil refining industry some 40 years ago. This 'low-tech' biological treatment method involves the controlled application and spread-out of a more or less defined organic bio-available waste (liquid, semi-solid or solid) on the soil surface and the incorporation of the waste into the upper soil zone. As a result of physical, chemical and biological processes occurring in the soil, the constituents in the applied waste are (bio)degraded, immobilized or transformed to environmentally acceptable components. This type of soil depollution focuses mainly on the biological breakdown of the applied organic waste by aerobic microorganisms in the top layer of soil where oxygen is adequately available. Metabolism in these microorganisms ultimatly decreases the organic pollutant to carbon dioxide, water and biomass.

In the last decade, investigations have shown that environmental factors enhancing soil microbial activity, such as fertilizing, ploughing and drainage, increase the breakdown of the organic pollutants (Vanloocke *et al.* 1979; Verstraete *et al.* 1980; Hoeks *et al.* 1988; Harmsen 1991). Furthermore, the application of inocula can optimize the landfarming technique (Atlas & Bartha 1973; Fusey & Oudot 1973; Westlake *et al.* 1976). In recent years, the landfarming method has gained increasing acceptance because it is a relatively simple and cheap method of cleaning soil contaminated with different organic compounds. Sludge farming activities are in operation in Flanders since 1987.

This study was conducted to evaluate if oil sludges can be disposed of by landfarming without causing environmental damage. In a first stage laboratory experiments were performed to investigate the feasibility of landfarming as remediation technology. In a second part field experiments were conducted to measure the actual biodegradation of the oil sludge and to analyze the effect of landfarming on the ecosystem. The objectives of this study were to (a) select the optimal landfarming treatment, (b) demonstrate that landfarm-

ing is an appropriate technology for the remediation of oil sludges; and (c) show that landfarming has no adverse effect on important environmental parameters such as groundwater quality, toxicity to soil organisms and plant growth.

## 2. Materials and methods

### 2.1. Laboratory experiments

#### 2.1.1. Soil sampling

The soil used in this study was collected during the summer season from a prospective landfarming site on the premises of the petroleum refinery Texaco at Ghent. After removal of surface litter, the soil was collected to a depth of 30 cm and passed through a 2 mm sieve to remove the larger roots, macrofauna and stones. Water was added to 2/3 of the field capacity (10% moisture). The samples were stored at 20 °C in plastic bags until use.

#### 2.1.2. Soil properties

For the physico-chemical analyses of the soil, the sample was air-dried and ground to pass a 2 mm sieve. The pH of the soil was measured in water using a 1:5 soil-water ratio. The clay percentage was obtained according to the pipette method (Kilmer & Alexander 1949). The organic carbon content was determined according to the Walkley and Black method (Bremner & Jenkinson 1960), and the cation exchange capacity (CEC) by means of the $NH_4Ac$-method (Schollenberger & Simon 1945). The total nitrogen content was determined according to the Kjeldahl method. The ammonium acetate-EDTA extraction was used to determine the 'nutrient' status of the soil (Cottenie *et al.* 1979).

#### 2.1.3. Extraction and separation of hydrocarbons

The hydrocarbon contents of the oil sludge and soil were measured gravimetrically after Soxhlet extraction (Dibble & Bartha 1979a). The chloroform extracted hydrocarbons were separated further into class fractions by silica gel column chromatography (Dibble & Bartha 1979a).

#### 2.1.4. Oil sludge

Oil sludge samples were collected from an open storage pit at the refinery Texaco. The sources of the material were oil-water separators, storage tank bottoms, and small spills on the refinery property. The moisture and ash content of the oil sludge were determined gravimetrically after respectively drying in an electric drying-kiln at 105 °C for 24 h and dry ashing in a muffle furnace at 650 °C for 2 h. The characteristics of the oil sludge are shown in Table 1.

*Table 1.* Characterization of the oil sludge.

| Parameter | value (%) |
|---|---|
| Moisture content | 32 |
| Ash content | 10 |
| Chloroform extractable HC | 44 |
| Silica gel fractionation | |
| Saturated | 36 |
| Aromatic | 51 |
| Asphaltic | 13 |

#### 2.1.5. Biodegradation experiments

Erlenmeyer flasks of 500 ml were loaded with 100 g of soil and 10 g of oil sludge containing 4.4 g of chloroform extractable HC. Mineral nutrients were added as $NH_4NO_3$ and $K_2HPO_4$ to give different initial carbon-mineral nutrient ratios. Control flasks received no mineral nutrient supplementation. Some flasks also received organic supplements, i.e., 0.4 or 2.0 g of dried compost per flask. Compost but no oil sludge was given to the control. Another series of flasks received only the mineral nutrients and 0.5 g of inoculum. The latter was prepared by mixing 100 g fresh oil with 1 kg of already oil contaminated soil together with fertilizers (1.5 g $NH_4NO_3$ and 0.5 g $K_2HPO_4$) and organic matter (4 g dried compost). The mixture had been allowed to develop for 14 days at 28 °C prior to being used. Table 2 gives an overview of the different treatments. All the erlenmeyer flasks were well mixed and then connected to a respirometer at 20 °C.

The soil respiration was determined by measuring the $CO_2$-production of the soil. Erlenmeyer flasks, containing soil (Table 2), were provided with small beakers (100 ml) containing 10 ml of distilled water to prevent dessication of the soil. Then the flasks were connected to the respirometer and flushed with $C0_2$-free air. Oxygen was continuously replenished by drawing air through a minicolumn fitted on top of the flasks.

The erlenmeyer flasks were connected to wash-flasks containing 5 ml alkali solution (0.5 N KOH). Alkali solutions in the flasks were removed every week and the amount of $CO_2$-produced was measured

*Table 2.* Overview of the different treatments.

| Treatment | | Mineral nutrients (mg/kg) | | |
|---|---|---|---|---|
| | | N | P | K |
| 1. | 100 g soil (blanc) | 0 | 0 | 0 |
| 2. | 100 g soil + 10 g oil sludge | 0 | 0 | 0 |
| 3. | 100 g soil + 10 g oil sludge | 256 | 53 | 134 |
| 4. | 100 g soil + 10 g oil sludge | 4000 | 445 | 1115 |
| 5. | 100 g soil + 10 g oil sludge + 0.4 g dried compost | 256 | 53 | 134 |
| 6. | 100 g soil + 10 g oil sludge + 2.0 g dried compost | 256 | 53 | 134 |
| 7. | 100 g soil + 10 g oil sludge + 0.4 g dried compost | 0 | 0 | 0 |
| 8. | 100 g soil + 0.4 g dried compost | 256 | 53 | 134 |
| 9. | 95 g soil + 10 g oil sludge + 5 g inoculum | 256 | 53 | 135 |

titrimetrically. The net amount of $CO_2$-produced in the treated soils corresponded to the amount of $CO_2$ formed in the soil sample minus the amount formed in the respectively control sample (treatments 1 and 8). The cumulative results were plotted for a testperiod of 140 days. All experiments were done in triplicate.

*2.1.6. Ames test*

The *Salmonella*/microsome assay of Ames *et al.* (1975) was used to evaluate the mutagenicity of the oil sludge. Therefore, the oil sludge and the soil sample of treatment 4 (Table 2) were extracted by shaking two times 10 g sample with respectively 90 ml of water and 90 ml of acetone for 12 hours. The test strain (0.1 ml of a culture in the exponential phase) was plated onto a glucose minimal medium and provided with 0.1 ml of a 0.5 mM histidine-biotine solution, 0.1 ml of the soil extracts and 2 ml agar layer. Controls received no soil extract but the same amount of water or acetone. Daunomycine and $NaN_3$ were used as control mutagenic compounds. The plates were incubated at 37 °C and after 72 hr the revertant colonies were counted and compared with the controls as an indication of the sample's mutagenicity.

*2.2. Field experiments*

A large-scale field experiment was started in 1987 to study the effects of landfarming.

*2.2.1. Test site*

The test site was located on the premises of the petroleum refinery Texaco at Ghent. It was covered by mixed grasses and annual herbaceous plants and was annually mowed until initiation of the project. It had not been used for agriculture and fertilizers nor pesticides had been applied for at least the last 20 years. The test area comprised about 2 hectare and provisions were made to capture surface run-off and drainwater. The latter included (a) digging of a draining ditch around the test site (0.5 m depth and 1.0 m width), (b) construction of a closed drainage system of 80 cm depth with a distance between the drain-pipes of 5 to 10 m; all the drainwater was collected in a sewerpit from where it was moved to a rainwater retention pond. Inside the test area the groundwater was monitored by analyzing samples taken from four wells of 8 m depth, located at every side of the landfarm.

*2.2.2. Method of oil sludge application and landfarming*

After excavation from the storage reservoir at the Texaco refinery, the oil sludge (40% HC) was mixed with soil from the upper 30 cm of the landfarm site, resulting in a sludge-soil mixture with a total volume of about 5,400 $m^3$. This mixture had a HC content of approximately 4.7%. At the onset of the landfarming (May 1987), 1,350 $m^3$/ha of the sludge-soil mixture was distributed over the field as homogeneously as possible to give a layer of about 15 cm. Fertilizers were added to levels corresponding with 500 kg N/ha, 100 kg P/ha and 50 kg K/ha, together with a starter-inoculum (10 kg/$m^2$). The inoculum was prepared by mixing 2 $m^3$ fresh oil sludge with 200 ton contaminated soil together with fertilizers (100 kg N, 20 kg P and 10 kg K) and organic matter (800 kg DW compost). This inoculum had been stored for about 2 months in a static pile prior to the start of the field experiment. Then, the sludge-soil mixture and the inoculum were ploughed into a depth of about 30 cm. Monthly tillage of the contaminated soil to a depth of 30 cm was applied to maintain good soil structure and to supply sufficient oxygen to the soil microbiota.

After 9 months, a second supply of 1,350 $m^3$ prehomogenised sludge-soil mixture was added per hectare of landfarming area. Once again, the soil was amended with nutrients to the levels mentioned above and mixed to a depth of 30 cm. Monthly tillage of the landfarm was continued till October 1991.

*2.2.3. Sampling and analysis*

Two forms of monitoring were used by the represented landfarming facility, namely operational and environmental monitoring. Sampling and analysis was done as described in Section 2.1. The operational monitoring was conducted periodically (not during winter) to evaluate soil characteristics in order to determine the breakdown of the applied hydrocarbons and the need for fertilizers. Environmental monitoring was conducted one to four times per year to evaluate subsurface soil and groundwater characteristics. Groundwater was monitored for indicator parameters such as pH, COD, HC content, $NH_4^+$-N and ($NO_2^-$ + $NO_3^-$)-N concentrations and specific conductance. These determinations were done as described by the American Public Health Association (1980).

*2.3. Ecotoxicological experiments*

*2.3.1. Soil*

To estimate the toxic effect of the residual HC in the landfarmed soil (soil A2), the soil was extracted with respectively cyclohexane (soil A3) or dichloromethane-methanol (1:1; v/v) (soil A4). The extracted soil was air-dried and restored to 10% moisture content by adding an appropriate amount of soil extract. The latter was prepared by shaking control soil (soil A1) with water in a ratio of 1/5 (w/w) for 12 hours. Afterwards, the mixture was filtrated and the filtrate was used. The control soil (soil A1) in these tests received no oil sludge.

*2.3.2. Ecotoxicological tests*

Worm tests, germination and plant growth tests were performed according to the OECD guidelines for testing of chemicals (1981). A series of other tests described in the literature to detect ecotoxic effects were used. The Rotox determines the effect of aquatic mixed cultures (Snell & Persoone 1989). The Microtox measures the impact on the luminescent aquatic bacterium *Photobacterium phosphoreum* (Bulich 1979). The Streptox determines the influence on *Streptocephalus proboscideus* (Centeno et al. in press). In these tests, water extracts were prepared by shaking 10 g soil with 90 ml testmedium EPA for 20 hr. After filtration of the suspension, the filtrate was centrifuged during 10 min at 2000 rpm. In these series of tests only the landfarmed soil (soil A2) and the control soil (soil A1) were examined.

*Table 3.* Physical and chemical properties of the soil used in this study.

| Properties | Value | Nutrients | Value (mg/kg DW) |
|---|---|---|---|
| % clay (0–2 $\mu$m) | 8.6 | $NH_4^-$-N | 1.15 |
| % silt (2–50 $\mu$m) | 13.2 | $NO_3^-$-N | 4.25 |
| % sand (50–2000 $\mu$m) | 78.2 | Total N | 980 |
| pH $H_20$ (1:5) | 8.17 | Total P | 14.3 |
| % OC | 0.94 | Na | 102 |
| CEC (meq/100 g DW) | 5.18 | K | 110 |
| | | Mg | 96 |

## 3. Results and discussion

*3.1. Laboratory experiments*

*3.1.1. Soil analysis*

The soil from the prospective landfarming site had a loamy sand texture (Table 3). Because of the poor soil quality indicated by the data in Table 3, the soil was conditioned before use in the biodegradation tests as describe in Section 2.1.5.

*3.1.2. Extraction of hydrocarbons*

The choice of the solvent used for the extraction and subsequent analysis of the hydrocarbons (HC) from the loamy sand soil was of special concern. The amount of HC extracted from the soil varied significantly with the solvent used. In other words, the residual HC level in a soil extracted with a less efficient solvent will be higher than in a soil extracted with a high efficient solvent. This had implications with regard to the evaluation of the residual levels of hydrocarbons as a function of time. Therefore, the efficiency of twenty solvents to extract the HC from the loamy sand soil after 5 years of landfarming was compared at the end of the field experiments (Table 4).

The singular solvents can be devided into three groups. Group 1 consisted of chlorinated solvents, such as dichloromethane, chloroform and tetra, which were the most effective in the removal of HC. They removed about 12 g/kg air-dried soil. An exception to this was freon 11 which extracted only 7.91 g/kg air-dried soil. Solvents of group 2, such as acetone, methanol and diethylether, which possess functional groups other than chloride were also very effective. They removed

*Table 4.* Extraction efficiency of twenty solvents to extract the HC from the loamy sand soil after 5 years of landfarming. The TLV-TWA value for each solvent is mentioned.

| Solvent | HC extracted[a] | TLV-TWA value[b] |
|---|---|---|
| Group 1 | | |
| Dichloromethane | 12.68 ± 1.860 | 50 |
| $CCl_4$ | 12.27 ± 0.530 | 5 |
| Chloroform | 12.22 ± 0.214 | 10 |
| Freon 11 | 7.91 ± 0.545 | 1000 |
| Group 2 | | |
| Diethylether | 11.47 ± 0.436 | 400 |
| Acetone | 11.28 ± 0.348 | 750 |
| Methanol | 19.75 ± 1.536 | 200 |
| Petroleumether | 7.94 ± 0.376 | – |
| Ethylacetate | 7.24 ± 1.094 | 400 |
| Group 3 | | |
| Iso-octane | 9.01 ± 0.415 | – |
| Hexane | 8.72 ± 0.498 | 50 |
| Benzene | 6.85 ± 0.903 | 10 |
| Cyclohexane | 4.96 ± 1.144 | 300 |
| Group 4 | | |
| Dichloromethane/methanol (v/v:1/1) | 14.54 ± 0.011 | 50/200 |
| Chloroform/methanol (v/v:1/1) | 14.51 ± 0.495 | 10/200 |
| Acetone/hexane (v/v:1/9) | 14.15 ± 0.539 | 750/50 |
| Acetone/pentane (v/v:1/1) | 13.45 ± 0.463 | 750/– |
| Petroleumether/ethylacetate (v/v:3/1) | 12.35 ± 0.274 | –/400 |
| Acetone/freon (v/v:1/1) | 12.03 ± 0.328 | 750/1000 |
| Methanol/water (v/v:4/1) | 9.22 ± 4.263 | 200/0 |

[a] mean value of three independent extractions of hydrocarbons (g/kg air-dried soil)

[b] threshold limit value and time weighted average (mg/l); the greater the value, the lower the toxicity.

about 10 g/kg air-dried soil, and were not significantly different ($P < 0.05$) from group 1 in the removal of the HC from the loamy sand soil.

The apolar solvents of group 3, such as cylcohexane and benzene, were the least effective. They removed about 7 g/kg air-dried soil, and were significantly different ($P < 0.05$) from group 1 in the removal of the HC from the loamy sand soil, but not from group 2. The combination of solvents (group 4; mean amount HC extracted: 12.9 g/kg air-dried soil) seemed most effective, but not significantly different ($P < 0.05$) from the singular solvents (group 1 + group 2 + group 3; mean amount HC extracted: 9.4 g/kg air-dried soil), in the removal of the HC from the loamy sand soil in a Soxhlet apparatus procedure.

In view of its low toxicity and high extraction effectiveness (Table 4) acetone was preferred as the most suitable solvent for the extraction of soils and subwater soils polluted with hydrocarbons. Acetone is also compatible subsequently with GC and HPLC techniques.

### *3.1.3. Optimization of oil sludge biodegradation*

In order to investigate the possibility of optimizing the biodegradation of oil sludge, soil was supplemented with varying quantities of nutrients, in combination with organic supplements and inoculum. Figure 1 shows the cumulative net $CO_2$-C evolution in the different treatments over a period of 5 months (140 days). Analysis of variance with factioral designs (time, treatment) indicated significant differences ($P < 0.05$) in the rate of soil respiration in the first three months. Treatment 4 (Table 2) reflects inhibition due to an overdosis of minerals. Treatment 2 and 7 received no additional N, P and K, and were clearly lower in mineralization than treatments 3, 5, 6 and 9 which were all enriched with mineral nutrients. Further, there was a positive effect of the compost (compare treatments 3, 5 and 6) and of the inoculum (compare treatments 3 and 9).

### *3.1.4. Ames test*

The mutagenic potential of the oil sludge before landfarming, and the soil of treatment 4 (Table 2) was monitored using *Salmonella*/microsome mutagenicity assay. The results are given in Table 5.

No differences were found in the number of revertants in the tests with the water and acetone extracts compared to their controls, though the two species of *S. typhimurium* were mutated by daunomycine and $NaN_3$ control. From this it can be concluded that the mutagenic potential of the HC extracted from the oil sludge and soil + oil sludge was low.

## *3.2. Field experiments*

### *3.2.1. Degradation of oil sludge*

The results of the HC degradation as well as the average monthly air temperature are given in Figure 2.

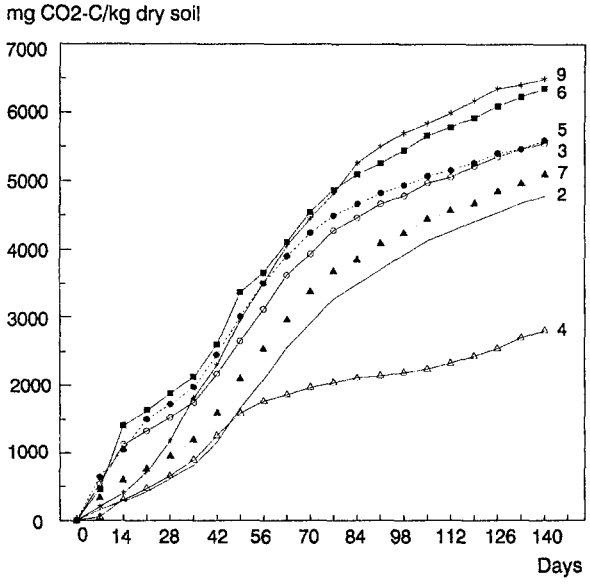


*Fig. 1.* Cumulative net $CO_2$-C evolution (mean of 3 replicates) during a testperiod of 140 days. The graph nummering corresponds with the treatment numbers described in Table 2. Treatment 1 was substracted as blanc from treatments 2, 3, 4 and 9; treatment 8 as blanc from treatments 5, 6 and 7 (see Table 2).

*Table 5.* Mutagenicity of oil sludge and soil + oil sludge (treatment 4) after 150 days of landfarming, as measured with *Salmonella typhimurium* TA98 and TA100. Daunamycine and $NaN_3$ are control mutagenic compounds.

| | Number of revertants | | | |
|---|---|---|---|---|
| | Oil sludge | | Soil + oil sludge | |
| Sample | TA98 | TA100 | TA98 | TA100 |
| Soil water extract | 13 ± 6 | 85 ± 14 | 17 ± 2 | 135 ± 6 |
| Water (control) | 7 ± 3 | 100 ± 22 | 10 ± 4 | 114 ± 8 |
| Soil acetone extract | 11 ± 4 | 68 ± 2 | 7 ± 2 | 103 ± 5 |
| Acetone (control) | 11 ± 3 | 83 ± 6 | 10 ± 2 | 113 ± 37 |
| Daunomycine | > 1000 | > 1000 | > 1000 | > 1000 |
| $NaN_3$ | > 1000 | > 1000 | 50 ± 2 | 214 ± 20 |

During the first period, June '87 – April '88, the oil was degraded fast from 23.1 g/kg dry soil to a residual concentration of 11.8 g/kg dry soil at an overall degradation rate of 15 g/kg dry soil per year. The HC degradation showed first order reaction kinetics. The best fit was obtained for the following equation ($R^2$ = 0.973, df = 4, P < 0.01).

$$\text{LnY} = 3.260 - 0.0823\,\text{X}$$

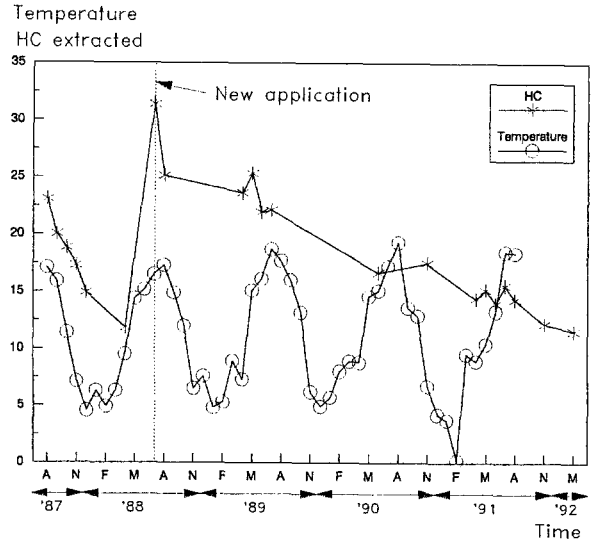


*Fig. 2.* Average HC concentrations (g/kg dry weight) and average monthly air temperature (°C) at the test area from August '87 till March '92.

With : Y = amount of HC extracted (g/kg dry soil);
X = time (months).

The second supply of 1,350 $m^3$ oil sludge increased the HC concentration to 31.4 g/kg dry soil. After 11 months, the HC were degraded to a concentration of 23.6 g/kg dry soil, which is equivalent to a degradation rate of 8.5 g/kg dry soil per year. In the subsequent 29 months, the HC concentration decreased slowly to 14.3 g/kg dry soil. In this period, the microbial activity decreased from about 60 to 5 mg $CO_2$-C/kg dry soil per day (results not shown). The residual HC fraction was further mineralized at an overall degradation rate of 3.8 g/kg dry soil per year. After approximately 5 years of landfarming, a concentration of 11.6 g/kg dry soil is reached. The HC degradation pattern during the second period also showed first order reaction kinetics. The best fit was obtained for the following equation ($R^2$ = 0.953, df = 15, P < 0.01).

$$\text{LnY} = 3.385 - 0.0195\,\text{X}$$

According to this equation, another 17 and 25 years, respectively, of landfarming will be necessary to reach a residual HC concentration of 500 mg (Dutch B-value) and 50 mg (Dutch A-value) of hydrocarbons.

In this study, it was not possible to statistically correlate the oil degradation to the average monthly air temperature. The effect of temperature was probably

*Table 6.* Silica gel fractionation of the oil sludge.

| HC-fraction | % Hydrocarbons (HC) | |
|---|---|---|
| | Aug '87 | March '92 |
| Saturated | 36 | 32 |
| Aromatic | 51 | 20 |
| Asphaltic | 13 | 48 |

masked by factors such as the variability of the samples and differences in soil moisture.

### 3.2.2. *Oil sludge analysis*

By silica gel column chromatography the hydrocarbon composition of the chloroform extracted fraction was devided into saturated, aromatic and asphaltic hydrocarbons. The data of the silica gel fractionation before (Aug '87) and after 5 years of landfarming (March '92) are given in Table 6.

During the landfarming, the fraction of aromatic hydrocarbons decreased strongly, whereas the asphaltic hydrocarbons proportionally increased. The amount of saturated hydrocarbons remained at about the same level. These results are in good agreement with the results of Dibble & Bartha (1979a). The expected degradation of the saturated fraction as measured by Raymond et al. (1976) was not observed. This could be explained by degradation of the aromatic compounds, of which produced intermediates are also saturated compounds, so that the overall degradation effect of the saturated compounds is nihil. The aspahaltic compounds are probably not degraded, but because of the degradation of the saturated and aromatic compounds they become a bigger fraction of the oil sludge.

### 3.2.3. *Leaching at field site*

The composition of the percolated water at the field site is given in Table 7.

The physical movement of oil, nitrogen and phosphate during the first 4 years of landfarming was minimal. The amounts of HC, nitrogen and phosphate in the percolate slightly increased after oil-sludge application. Analysis of variance with factorial designs (time, well) showed that there are no significant differences between the different sampling places and times. Table 8 shows that after 16 months of landfarming most of the HC remained in the top 40 cm, corresponding with the working depth of the cultivator that mixed the

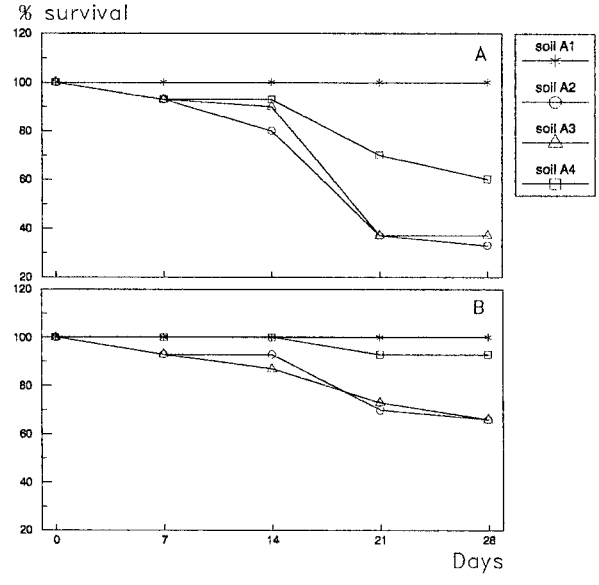


*Fig. 3.* Mean percentage survival of *Dendrobaena veneta veneta* (A) and *Eisenia foetida andrei* (B) after exposure to soil A1, A2, A3 and A4.

sludge into the soil to a depth of 30 cm. These results are in comparison with those of Hoeks et al. (1988), who observed oil migration to a depth of maximally 80 cm after 16 months of landfarming.

The pH of the percolated water was decreased less than 0.5 unit during the landfarming. The more or less constant pH was probably due to the buffer capacity of the landfarmed soil and the applied oil sludge. In this study, it was not possible to statistically correlate the migration of oil, nitrogen and phosphate with the rainfall (data not shown).

## 3.3. *Ecotoxicological experiments*

### 3.3.1. *Worm responses*

Two species *Dendrobaena veneta veneta* and *Eisenia foetida andrei* have been used in this study. The effect of the residual HC concentration on the survival of the two worm species was respectively tested in control soil (soil A1), landfarmed soil (soil A2), landfarmed soil extracted with cyclohexane (soil A3), and landfarmed soil extracted with dichloromethane-methanol (1:1; v/v) (soil A4). Cyclohexane removed 4.96 g HC/kg dry soil and dichloromethane-methanol 14.54 g HC/kg dry soil (Table 4). The effects are presented in Figure 3.

*Table 7.* Average values of the indicator parameters in the percolated water from Aug '87 – Dec '91.

| Date | pH | HC (mg/l) | COD (mg/l) | $NH_4^+$-N (mg/l) | $(NO_2^- + NO_3^-)$-N (mg/l) | $P_{tot}$ ($\mu$ g/l) |
|---|---|---|---|---|---|---|
| Aug '87 | 6.5 | 14.4 | 44 | 3.2 | 2.1 | – |
| Apr '88 | 6.9 | 25.1 | 32 | 0.6 | 1.9 | 92 |
| Oct '88 | 6.7 | n.d. | 69 | 2.6 | n.d. | 210 |
| Apr '89 | 6.6 | n.d. | 52 | 1.2 | 1.1 | 182 |
| Nov '89 | 6.6 | 6.0 | 76 | 2.1 | n.d. | 28 |
| Jan '90 | 6.2 | n.d. | 57 | n.d. | n.d. | 108 |
| Nov '90 | 6.9 | 21.0 | 76 | 2.1 | 0.1 | 5 |
| May '91 | 6.4 | 1.7 | 88 | 1.8 | n.d. | – |
| Aug '91 | 6.2 | 8.0 | 50 | 1.8 | 0.5 | – |

n.d. = not detected; – = not executed

*Table 8.* Concentration of HC in the land-farmed soil at various depths after 16 months of landfarming.

| Depth (cm) | HC content (g/kg dry soil) |
|---|---|
| 0 - 20 | 15.2 |
| 20 - 40 | 20.6 |
| 40 - 60 | 7.9 |
| 60 - 80 | 1.9 |
| 80 - 100 | 0.04 |

*Table 9.* Effect of HC on the germination of garden cress and seakale beet.

| Soil | Percent Germination | |
|---|---|---|
| | Garden cress (n=50) | Seakale beet (n=20) |
| A1 | 95 ± 4 | 78 ± 22 |
| A2 | 95 ± 4 | 65 ± 5 |
| A3 | 95 ± 5 | 65 ± 5 |
| A4 | 89 ± 6 | 68 ± 2 |

n = number of seedling

Both worm species showed a more or less similar response. After a 28-day exposure of the worms to the control soil (soil A1), no mortality was registrated; the earthworm biomass had decreased with 15%. In the land-farmed soil (soil A2) an increased mortality of the worms was found. Yet, in the soils A3 and A4, in which most the hydrocarbons were removed by extraction, a similar effect was noted. The latter suggests that probably salts or other compounds of the oil sludges, rather than the HC per se, caused the residual effects. These results are in agreement with those of Neuhauser *et al.* (1989) and Loehr et al. (1992) who reported that residual oil concentration had no significant effect on earthworms.

*3.3.2. Effects of the residual HC on germination*

In order to detect the effect of the residual HC concentration on germination of garden cress and seakale beet, germination was followed in soil A1, A2, A3 and A4. The results of this test are given in Table 9.

The residual HC in the loamy sand soil had no effect on the germination of garden cress and seakale beet. A same amount of seedlings had emerged in the control soil (soil A1), the polluted soil (soil A2) and the extracted soils (soils A3 and A4). These results are comparable with those of Dibble & Bartha (1979b) who observed no germination inhibition with wheat and soybeans in kerosine contaminated soil.

*3.3.3. Effects of the residual HC on plant growth*

In order to study possible chronic effects of the residual HC concentration on plants, growth of corn and seakale beet in soil A1, A2, A3 and A4 was followed (Table 10).

After 21 days of growth, no differences in dry weight were measured for the corn plants in the different treated soils, i.e., the presence of the residual HC

*Table 10.* Effect of HC on the growth of corn and seakale beet after 21 days of growth.

| Soil | Dry weight (g) | |
|---|---|---|
| | Corn | Seakale beet |
| A1 | 1.05 ± 0.05 | 0.48 ± 0.01 |
| A2 | 1.04 ± 0.01 | 0.24 ± 0.05 |
| A3 | 0.90 ± 0.18 | 0.13 ± 0.02 |
| A4 | 1.16 ± 0.25 | 0.31 ± 0.03 |

*Table 11.* Effects of the eluates from the landfarmed soil and the control soil in three ecotoxicity tests.

| Test | Soil | |
|---|---|---|
| | Landfarmed soil (A2) | Control soil (A1) |
| Rotox | 10% mortality at 100% eluate | 10% mortality at 100% eluate |
| Streptox | 20% mortality at 100% eluate | 0% mortality at 100% eluate |
| Microtox | stimulation | not toxic |

was not inhibitory. On the contrarary, the growth of the beet plants in the contaminated soil (soil A2) appeared stunted compared to the plants in the non-contaminated soil (soil A1). As the plants received supplementary nutrients, the reduced growth was not a result of competition with hydrocarbonoclastic microorganisms for mineral nutrients. As with the worm tests, the negative effect of the HC could only in part be removed by subjecting the soils to an extensive extraction procedure (soil A4). Again, some other components of the oil sludge rather than the hydrocarbons appeared to exhibit phyto-inhibitory effect.

*3.3.4. Other tests*

Three species of microorganisms, namely *Brachionus calyciflorus* (Rotox), *Streptocephalus proboscideus* (Streptox) and *Photobacterium phosphoreum* (Microtox), have been used in this study to evaluate the ecotoxicity of the different eluates. Table 11 gives an overview of the registered effects.

These ecotoxicological tests indicated that the eluate of the landfarmed soil had no ecologically significant acute toxicity. Only the mortality in the Streptox-test exceeded the threshold limit value of 10%, but the ecologically significant value of 50% mortality was never reached, not even by 100% eluate. The bioluminiscence as measured in the Microtox-test, neither indicated any sign of adverse effects.

## 4. Conclusions

Laboratory experiments were conducted with the aim of optimizing the environmental parameters of 'landfarming', i.e., the disposal by biodegradation in soil of oil sludge generated in the petroleum refinery Texaco at Ghent. The addition of mineral nutrients resulted in an increased biodegradation. Above a certain level, nutrient addition resulted in inhibition. The additon of compost as organic supplement and a bacterial inoculum had a positive effect on the biodegradation.

The results of the field experiments indicated that soil has the capacity to treat oil sludges. During the first 12 months after sludge application the degradation of HC were degraded at a rate of 15 g HC/kg dry soil per year. The following 40 months the degradation rate decreased to approximately 4 g HC/kg dry soil per year and the microbial activity decreased from about 60 to 5 mg $CO_2$-C/kg dry soil per day. Analysis of the residual hydrocarons by component classes showed that the breakdown of the aromatic compounds was the highest and that asphaltic compounds were the least susceptible to biodegradation. According to the obtained first order kinetics oil sludge can effectively be treated if a sufficient time (minimum 15 years) for bioremediation is provided.

The Ames test showed no mutagenicity of the oil sludge and of the landfarmed soil. During the first four years of landfarming the migration of oil, nitrogen and phosphate was minimal. Most HC remained in the top 40 cm, which was the working depth of the cultivator. Extensive efforts with worm test, germination test and plant growth test showed no adverse impact of the residues remaining in the soil from biodegradation of oil sludge. In some cases, probably salts or other compounds of the oil sludges caused residual effects.

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