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Risk assessment for residual mineral oil contaminants in bioremediated soil

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table of contents

summary

Mineral oil products (e.g., diesel fuels, and heating oils) are complex mixtures, mainly consisting of petroleum hydrocarbons covering a wide range of physico-chemical properties. Due to leakage (e.g., underground storage tanks, and pipelines), periodic spills (e.g., refueling facilities) and accidental spills (e.g., transport accidents), these mineral oil products can contaminate the soil. Bioremediation is a promising and gentle remediation technique to treat mineral oil contaminated soils. During the remediation activities, the total contaminant concentration decreases and the composition of the initial contaminants changes. However, after the treatment has been completed, a complex mixture of organic contaminants remains in the soils, which is commonly determined as *Total Petroleum Hydrocarbon* (TPH). While the chemicals of concern in fresh mineral oil

products (e.g., PAHs, BTEX) are very well investigated and regulated, very little information exists on the TPH mixture in bioremediated soils. Due to the lack of knowledge regarding the potential environmental hazards associated with these contaminants, the soils often have to be disposed of in landfills which is unsatisfactory from both, an economic and ecological point of view.

In this work, emphasis was put on the physicochemical characterization and the environmental behavior of these TPH components as a basis to perform a risk assessment. A case study, including field and laboratory experiments, was conducted using bioremediated soil which was obtained from a remediation company after the treatment had been completed. The analytical concept employed in the *hazard identification* revealed that all of the residual contaminants within the bioremediated soil must possess a very low mobility in the environment due to their low volatility as well as their high hydrophobicity ($K_{ow} > 10^6$). In the *exposure assessment*, the relevant transport and transformation processes were identified and quantified. Furthermore, a mass-flow analysis was performed for the residual contaminants in the bioremediated soil. It is indicated that the majority of the residual contaminants (> 90%) will remain in the soil after one year of soil application as top soil. The amount of total solvent extractable material (< 10%) which were lost in this time period could be further divided into different processes: 98% of the total losses were due to transformation processes, a combination of biodegradation and aging effects (chemical oxidation reactions incorporating the contaminants into natural organic matter; slow diffusion of the contaminants into very small pores); volatilization and plant uptake were estimated to be negligible; and leaching, although identified as the major transport process, accounted for only little removal (1.7 % of the losses). It can therefore be concluded that only very low emissions can be expected from this soil. The *toxicity assessment*, including laboratory experiments as well as model calculations, indicated that the residual contaminants in the bioremediated soils do not cause any environmental hazards, neither to water organisms nor to plants. As a result of the *risk assessment*, the bioremediated soil investigated in this case study could be widely reused, even for

applications where a receptor would be exposed by several pathways (e.g., as top soil in residential areas).

Based on the data presented in this study, an alternative method to the presently used approach for the evaluation of bioremediated soils and mineral oil contaminated soils, in general, is presented. Rather than evaluating just one surrogate parameter (TPH) in soils, the potential risk associated with the residual contaminants therein must be determined, based on its potential emissions.

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Zusammenfassung

Mineralölprodukte wie Dieseltreibstoff oder Heizöl sind komplexe Gemische von zahlreichen organischen Verbindungen. Hauptsächlich bestehen sie aus Kohlenwasserstoff-Verbindungen, welche die unterschiedlichsten chemisch-physikalischen Eigenschaften besitzen. Durch Leckagen von Tanks oder Pipelines, durch Verluste bei Umfüllstationen sowie durch Transportunfälle können solche Verbindungen in den Boden gelangen und diesen verunreinigen. Eine vielversprechende Möglichkeit, das verunreinigte Bodenmaterial zu reinigen ohne dabei die Bodenstruktur zu zerstören, stellen biologische Sanierungsverfahren dar. Mit Hilfe von natürlich vorkommenden Mikroorganismen im Boden wird ein Grossteil diese Verbindungen mineralisiert, doch trotzdem bleiben auch nach einer abgeschlossenen Sanierung gewisse Restschadstoffe im Bodenmaterial zurück.

Diese unterscheiden sich in Ihrer Zusammensetzung stark von den Ausgangsverbindungen. Diese Restschadstoffe werden meist als Summenparameter, den sogenannten *Total Petroleum Hydrocarbons* (TPH), quantifiziert. Während die als relevant ausgewählten Verbindungen in frischen Mineralölprodukten (BTEX, PAK) sehr gut untersucht und gesetzlich geregelt sind, bestehen nur sehr beschränkte Informationen über das Umweltverhalten und die Toxizität solcher Restschadstoffe. Um das Vorsorgeprinzip in der Gesetzgebung nicht zu verletzen, muss saniertes Bodenmaterial meist deponiert werden.

Das Ziel dieser Arbeit war es, diese Restschadstoffe chemisch-physikalisch zu charakterisieren sowie deren Umweltverhalten zu beschreiben um anschliessend eine Risikobewertung für die im sanierten Bodenmaterial verbleibenden Verbindungen zu erstellen. In einer Fallstudie wurde saniertes Bodenmaterial nach erfolgter Sanierung mit einer Reihe von Feld- und Laborexperimenten eingehend untersucht. Durch die chemisch-physikalische Charakterisierung der Restschadstoffe unter Verwendung von unterschiedlichen analytischen Methoden konnte gezeigt werden, dass die Mobilität dieser Verbindungen in der Umwelt aufgrund ihrer geringen Flüchtigkeit und ihrer hohen Hydrophobizität ($K_{ow} > 10^6$) äusserst gering sein muss. Die anschliessende Untersuchung der verschiedenen Transport- und Transformationsprozesse im Boden sowie die Berechnung der entsprechenden Massenflüsse ergab, dass der überwiegende Teil der Restschadstoffe (> 90%) auch nach einem Jahr nach der Wiederverwendung des untersuchten Bodenmaterials als Oberboden in der Bodenmatrix verblieb. Die geringen Verluste (< 10%) konnten verschiedenen Prozessen zugeordnet werden: 98% davon sind Verluste durch Transformationsprozesse im Boden selbst, durch biologischen Abbau sowie sogenannte Alterungsprozesse der Restschadstoffe (chemischer Einlagerung in die natürlichen, organischen Bestandteile des Bodens, die langsame Diffusion in sehr enge Porenräume sowie die Absorption an die Festphase). Berechnungen ergaben, dass nur vernachlässigbar kleine Mengen in die Atmosphäre gelangen oder durch Pflanzen aufgenommen werden. 1.7 % der Gesamtverluste gelangten ins Sickerwasser. In Toxizitätsstudien mit Wasser-

organismen sowie Wachstumsexperimenten mit Pflanzen konnten keine negativen Auswirkungen auf die Umwelt festgestellt werden. Basierend auf den experimentellen Daten sowie ergänzenden rechnerischen Abschätzungen wurde eine Risikobewertung für die Restschadstoffe im biologisch saniertem Bodenmaterial durchgeführt, mit dem Resultat, dass das untersuchte, biologisch sanierte Bodenmaterial als *uneingeschränkt wiederverwendbar* eingestuft werden konnte.

Aufgrund der Erkenntnisse dieser Untersuchung wird ein alternatives Vorgehen zu bestehenden Beurteilungsmethoden von biologisch saniertem als auch mineralölbelastetem Bodenmaterial vorgeschlagen. Um dieses Bodenmaterial umfassend beurteilen zu können, sind anstelle der Gesamtgehalte im Boden (TPH) selbst sämtliche potentielle Emissionspfade, die zu einer Verlagerung bzw. Verbreitung der Restschadstoffe führen könnten, einzeln zu untersuchen.

1

introduction

The awareness that chemicals and hazardous wastes in soils could cause serious environmental problems has grown in the last few decades. Particularly in cases of recent soil pollution, mineral oil products are among the major contaminants [Holliger and Zehnder, 1996; Thalmann and Küng, 1997]. In Switzerland, for example, in more than 60 % of environmental damages with liability claims between 1985 and 1993, mineral oil products were involved [Hoffmann, 1995]. There are several sources of how these mineral oil products could contaminate soils: leaking underground storage tanks, pipelines, landfills and dump sites as well as periodic spillage at industrial and refueling facilities and accidental spills during transportation [Mackay, 1988]. Gravity subsequently causes a migration of the contaminants downward through the unsaturated soil as a distinct fluid (Figure 1-1). The behavior of the hydrocarbon mixture in the soil is dependent on its composition, the porosity and moisture content of the soil, the prevailing temperatures and weathering conditions, the contour of the land, and the depth to the groundwater table. Low-viscosity mineral oil products

(gasoline, kerosene, diesel fuel, and heating oil) infiltrate into the soil rapidly and deeply, unless a very high texture, water saturation, or frozen state of the soil prevent this [Atlas and Bartha, 1992]. More viscous mineral oil products such as lubricating oils infiltrate into soil much more slowly and stay close to the surface of the contaminated soil [Atlas and Bartha, 1992].

Soil which has been polluted by mineral oil products can potentially cause a risk to surrounding environmental media due to further distribution of these chemicals, such as dissolution in groundwater and subsequently in drinking water, evaporation into the air, plant uptake and other processes. In order to prevent the spreading of contaminants, the soil must be remediated.

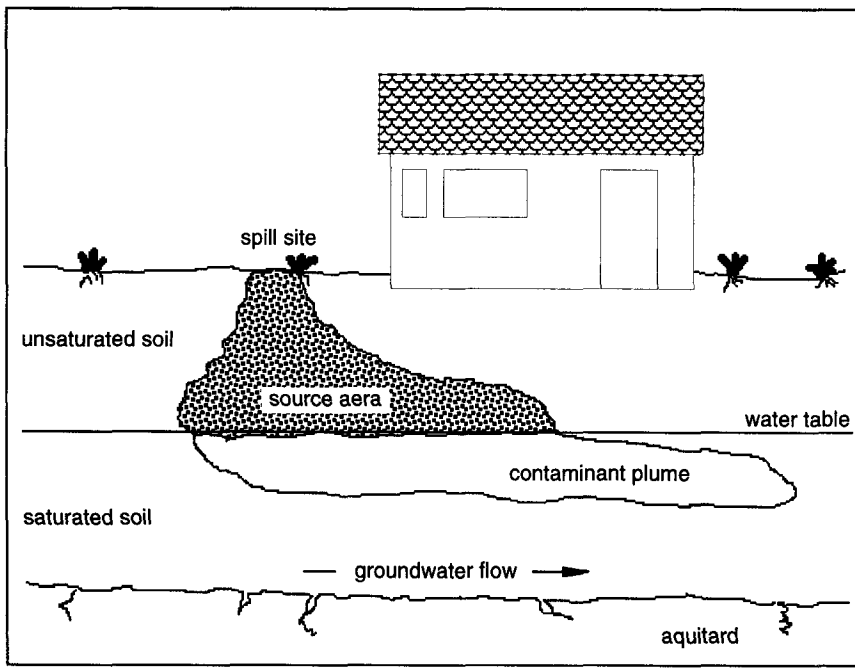

Figure 1-1 *Illustration of a contaminated site.*

1.1 Composition of mineral oil products

Mineral oil products are obtained from crude oils through different refinery processes which are described by Harms [1989]. According to a general statistics on energy consumption, 12 Mio. tons of mineral oil products are distributed and consumed per year in Switzerland. Approximately 50 % thereof are fuels (gasoline, kerosene, diesel fuel) and the other 50 % are heating oils [Bundesamt für Energiewirtschaft, 1996]. In addition, motor oils and lubricating oils are also used but in much lower quantities.

Table 1-1 *Detailed analyses of the composition of selected mineral oil products*

Hydrocarbon type	Gasoline [1] [% v/v]	Kerosene [2] [% v/v]	Diesel fuel / Heating oil [3] [% v/v]
Aliphatics			
n-Alkanes	11.0	57.0	22.2
iso-Alkanes	45.5		19.1
Alkenes	6.5		nd
Naphtenes			
Mono-cyclic alkanes	6.1	28.7	22.1
Bi-cyclic alkanes	nd [4]	3.4	9.6
Tri-cyclic alkanes	nd	1.8	2.3
Aromatics			
(Alkyl) Benzenes	28.8	8.5	5.9
Indanes/tetralins	0.7	nd	4.1
Dinaphthenobenzenes/indenes	nd	nd	1.8
Naphthalenes	1.4	0.6	8.2
Biphenyls/acenaphthenes	nd	nd	2.6
Fluorenes/ acenaphthylenes	nd	nd	1.4
Phenanthrenes	nd	nd	0.7

1) *adapted from Peterson [1994]*
2) *adapted from Riser-Roberts [1992]*
3) *adapted from Millner et al. [1992]*
4) *not determined*

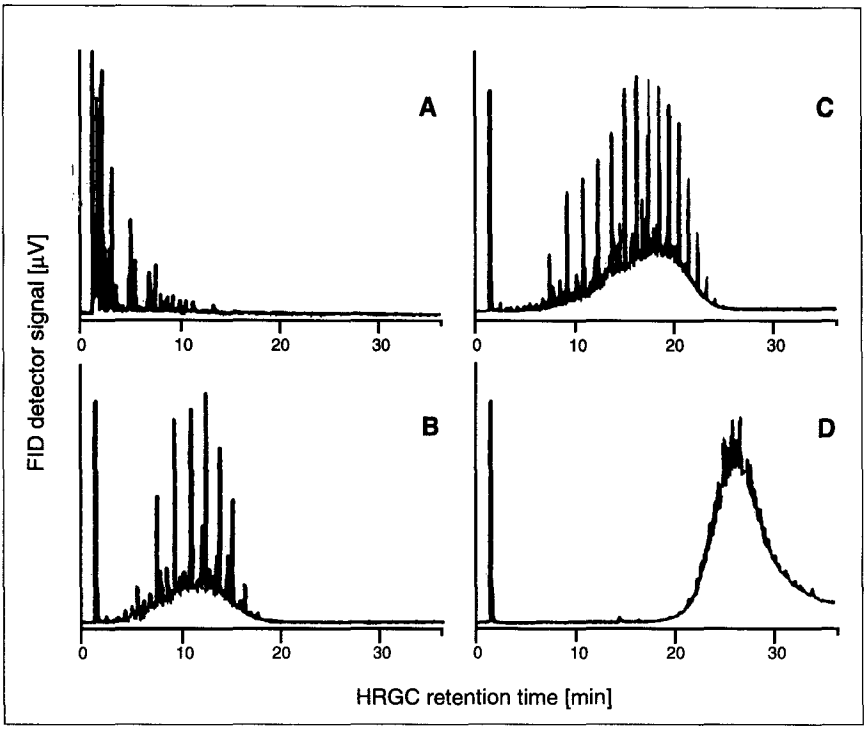

Figure 1-2 *Illustrative HRGC chromatograms of different mineral oil products: A) gasoline; B) kerosene; C) diesel fuel; D) lubricating oil, adapted from Zemo et al. [1995].*

Mineral oil products are complex mixtures of primarily petroleum hydrocarbons, covering a wide range of physicochemical properties. The composition of the mineral oils can vary according to the origin of the crude oil as well as the refinery processes [Harms, 1989]. In order to illustrate this complexity, the composition of some mineral oil products is presented in Table 1-1 and typical analytical information of unweathered mineral oil products, obtained by High Resolution Gas Chromatography (HRGC), is shown in Figure 1-2. The range of HRGC retention times and consequently the boiling range of the corresponding constituents increase in the

following order: *gasoline < kerosene < diesel fuel < motor oil.* A variety of distinct signals are present in the chromatograms from early to middle distillates. In the chromatograms from middle to late distillates, an increasing amount of the constituents remain unresolved and subsequently form an unresolved signal commonly referred to as *hump* [Zemo et al., 1995].

1.2 Soil remediation technologies

Different remediation technologies have been developed for the treatment of mineral oil contaminated soils. An overview of the different technologies is presented by Wille [1993] and summarized in Table 1-2.

Thermal treatment can be used for organic contaminants in the soil with a high degree of effectiveness. While the contaminants are destroyed within a relatively short treatment time, the energy consumption as well as the treatment costs are relatively high and the soil as a living system is destroyed. The effectiveness of *soil washing* is depending on the soil structure (not very effective for loam- and clay-rich soils) and the contaminant concentration. The treatment time is relatively short. However, the contaminants are not destroyed but concentrated to a smaller fraction which needs further treatment (e.g. disposal in landfills). *Immobilization* is a technology to solidify the soil which can not be treated by soil washing or bioremediation. The mobility of the contaminants is reduced but the contaminants themselves are not destroyed. Furthermore, the soil structure is changed due to a highly reduced soil porosity. *Bioremediation* is a technique which is stimulating processes which are also naturally occurring in soils, referred to as weathering processes [Bregnard et al., 1996]. It can be distinguished between *ex situ (on site; off site)*, and *in situ* bioremediation techniques, depending on the treatment location. While *in situ* remediation techniques treat the contamination directly at the location of its occurrence, the other techniques require that the contaminated soil is excavated before treatment at the same location (*on site*) or at a different place (*off site*). Bioremediation is the only soil treatment technology where the soil as a living system remains preserved and therefore is considered as a gentle soil remediation

technique. For the economic applicability of bioremediation, the lower costs, the lower energy consumption and, as long as autochthonous organisms are employed [Thalmann and Küng, 1997], the higher acceptance in the public are relevant [Wille, 1993]. Compared to the treatment duration of the alternative technologies listed in Table 1-2, however, the degradation processes for petroleum hydrocarbons in soils are slow. The majority of the mineral oil contaminants are mineralized but, depending upon the soil conditions and the hydrocarbons present in the soils, approximately 10 to 30 % of the initial contaminant concentrations have been found to remain in bioremediated soils [Wille, 1993; Miethe, 1994]. The multitude of these unidentified residual compounds usually are referred to as the Unresolved Complex Mixture (UCM).

Table 1-2 *Remediation technologies for mineral oil contaminated soils. Data adopted from Wille [1993]*

Technology	Treatment costs	Energy consumption	Remediation time	Destruction of contaminants	Preservation of soil fertility
Thermal treatment	high	high	short	yes	no
Physical treatment (e.g. soil washing)	medium	medium	short	no	no
Immobilization	medium	medium	medium	no	no
Bioremediation	low	low	long	yes [1)]	yes

[1)] *the majority of the contaminants will be mineralized, with some contaminants remaining in the soil [Miethe, 1994; Wille, 1993]; see below*

1.3 Factors affecting bioremediation

The biodegradation of crude oil and mineral oil products in the environment is influenced by various factors including: i) the nature and the amount of the hydrocarbons present, ii) the environmental conditions, and iii) the composition of the autochthonous microbial community.

1.3.1 The contaminants

It is reported in the literature that microorganisms in soils are able to degrade a variety of petroleum hydrocarbons, although at very different rates [Atlas, 1984; Kästner, 1993; Holliger and Zehnder, 1996; Bregnard et al., 1996 and 1998]. The relative degradation rates of these hydrocarbons under aerobic conditions, adapted from Kästner [1993], are:

n-alkanes (C_{10}-C_{19}) > n-alkanes (C_5-C_9) > iso-alkanes (<C_{12}) > alkenes (C_3-C_{11}) > iso-alkenes (<C_{12}) > monoaromatics (BTEX) > cycloalkanes > iso-alkanes (>C_{12})

It is reported that n-alkanes containing up to 44 carbon atoms (tetratetracontane) could be metabolized by microorganisms [Haines and Alexander, 1974]. Branched alkanes are less degradable than straight-chain alkanes and hydrocarbons with numerous quaternary C-atoms, such as heptamethylnonane, can be completely resistant to biodegradation [Kästner, 1993].

During the biodegradation processes, the initial contaminant concentrations in the soil decrease. As a result, the chemical composition and, as a consequence, the physicochemical properties of the remaining mixtures in the soils change [Millner et al., 1992]. In order to illustrate these processes, HRGC chromatograms for a diesel fuel in soil after different residence times are presented in Figure 1-3. The dominant n-alkanes typically found in unweathered diesel fuel with numbers of carbons between C_{10} and C_{20} are present as distinct signals. The iso-alkanes pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane)

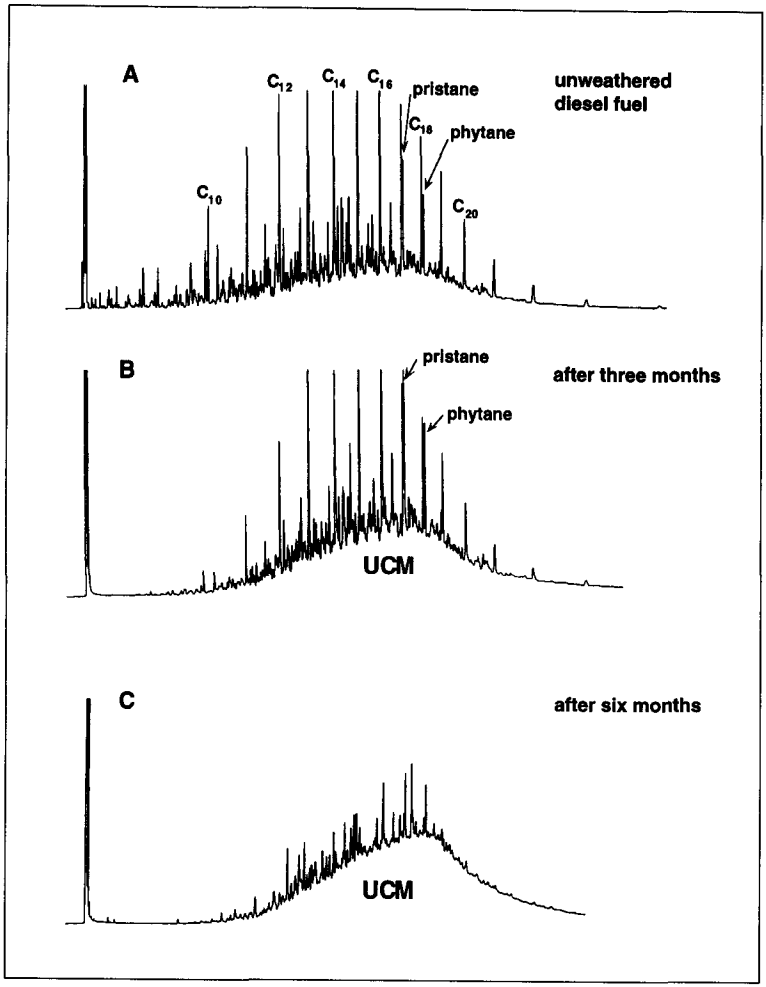

Figure 1-3 *The change in chemical composition of A) unweathered diesel fuel; B) weathered diesel fuel after three months; C) weathered diesel fuel after six months in soil is demonstrated by HRGC chromatograms with unequal resolution; adapted from [Eastcott, 1989].*

are also present in small amounts. In Figure 1-3 B, after three months of weathering, the components at lower HRGC retention times are lost, presumably due to a combination of evaporation and degradation processes. n-Alkanes are partially degraded, as it is indicated by the reduced signals (e.g. C_{12}) and moreover by the changed ratios between C_{17} and C_{18} and pristane and phytane, respectively. Figure 1-3 C shows weathered diesel fuel in soil after a residence time of six months, clearly indicating that many of the formerly dominating compounds, including pristane and phytane, have been degraded.

The degradation rate of a certain mineral oil contaminant in the soil is dependent on: i) the soil conditions, ii) the microorganisms present in the soil, and iii) the resident time of the chemicals in the soil. Mass transfer very often is the limiting factor in contaminated soils and consequently, increased microbial conversion capacities do not lead to higher biotransformation rates in these soils [Bosma et al., 1997]. The *bioavailability* of a chemical is controlled by a number of physicochemical processes such as sorption and desorption, diffusion and dissolution [Luthy et al., 1994; Karimi-Lotfabad et al., 1996; Bosma et al., 1997]. Particularly in old polluted sites, part of the contaminants appear to be inaccessible for biodegradation. The decrease of the bioavailability in the course of time is often referred to as *aging* [Bosma et al., 1997]. Aging effects may result from i) chemical oxidation reactions incorporating them into natural organic matter [Burgos et al., 1996; Karimi-Lotfabad et al., 1996], ii) slow diffusion into very small pores and absorption into organic matter, or, iii) if non-aqueous-phase liquids (NAPL) are present in soil, the formation of semi-rigid films around the NAPL with a high resistance toward NAPL-water mass transfer [Luthy et al., 1994]. Therefore, a reduced bioavailability of pollutants in soil is caused by the slow mass transfer to the degrading microorganisms and pollutants can become unavailable when the rate of mass transfer is zero (e.g. in the case of bound residues) [Bosma et al., 1997]. According to De Jonge et al. [1997], bioavailability was controlled by dissolution into the aqueous phase at high contaminant concentrations. At lower concentrations ($< 4 g \cdot kg^{-1}$), however, desorption and diffusion become rate-limiting factors. In the case of hydrocarbon contamination in soil, the toxicity to microorganisms is rarely a problem [Atlas and Bartha, 1992]. However, it is suggested that a maximum or threshold concentration of these contaminants in soil ecosystems exists [Leahy and Colwell, 1990], since the CO_2 evolution in soils

decreased at contaminant concentrations above 10 % (w/w) [Dibble and Bartha, 1979].

1.3.2 *Environmental conditions*

Efficient biodegradation is dependent on basic environmental factors such as nutrients, oxidants, temperature, pH, and moisture.

Temperature : Temperature influences hydrocarbon degradation by its effects on the physical nature and chemical composition of the oil, the rate of metabolism by microorganisms, and the composition of the microbial community. At low temperatures, the viscosity of the oil increases, the volatilization of toxic short-chain alkanes is reduced, and their water solubility increases [Leahy and Colwell, 1990]. Higher temperatures increase the rates of hydrocarbon metabolism to a maximum, typically in the range of 30 to 40°C [Yeung et al., 1997], above which the membrane toxicity of hydrocarbons is increased [Bossert and Bartha, 1984].

pH, moisture : Most heterotrophic bacteria and fungi favor a pH near neutrality, with fungi being more tolerant to acidic conditions [Bossert and Bartha, 1984]. Extremes in pH, as can be observed in some soils, would therefore be expected to have a negative influence on the ability of microbial populations to degrade hydrocarbons [Leahy and Colwell, 1990]. Furthermore, hydrocarbon degradation in terrestrial ecosystems can be limited by the available water for microbial growth. It is reported that optimal rates of biodegradation of oil sludge in soil result at 30 to 90% water saturation [Dibble and Bartha, 1979].

Nutrients : As reviewed by Bossert and Bartha [1984], contradictory results have been published concerning the influence of the addition of nutrients on the degradation rates of hydrocarbons in soil. While some authors reported immediate and strong stimulation on hydrocarbon degradation in contaminated soil after the addition of nitrogen and phosphorus salts, others found very little or no positive effects at all. These unequal responses could be explained by different nutrient pools which can exist in soils. Undoubtedly, nutrients must be present in soil. However, they must be in a form which is available for microorganisms.

Oxidants : Excellent reviews exist on the aerobic [Atlas, 1981; Bossert and Bartha, 1984; Leahy and Colwell, 1990; Atlas and Bartha, 1992] and anaerobic [Holliger and Zehnder, 1996] degradation of aliphatic and aromatic hydrocarbons. Compared to aerobic processes, the degradation rates found for these contaminants during anaerobic treatment processes with nitrate, Fe(III), sulfate, and carbon dioxide as electron acceptors are very low [Atlas, 1981; Leahy and Colwell, 1990; Holliger and Zehnder, 1996].

By optimizing the environmental conditions in the soil, the growth conditions for the microorganisms therein and subsequently the degradation rate of the contaminants are improved during a bioremediation process. For example, an *ex situ* soil remediation usually takes place in a tent where water and nutrients are added to the soil. Furthermore, the soil is aerated either by forced aeration or by tilling the soil periodically, and organic structure material (e.g. straw, bark) is added.

1.3.3 Microbial community

Hydrocarbons in the environment are biodegraded primarily by bacteria and fungi which are ubiquitous in terrestrial and aquatic ecosystems [Atlas, 1981]. The extent to which bacteria and fungi participate in the degradation of hydrocarbons has been the subject of only limited study, but appears to be a function of ecosystem and local environmental conditions [Leahy and Colwell, 1990]. Individual organisms can metabolize only a limited range of hydrocarbon substrates, so that assemblages of mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil and mineral oil products in soils [Leahy and Colwell, 1990]. After hydrocarbon spills in soils, the organisms must become adapted to the changed environmental conditions in order to be able to biodegrade these contaminants. There are three interrelated mechanisms by which adaptation can occur: i) induction and/or depression of specific enzymes, ii) genetic changes which result in new metabolic capabilities, and iii) selective enrichment of organisms able to transform the components of interest [Leahy and Colwell, 1990].

Compounds which otherwise would not be degraded can, incidentally and incompletely, be transformed within a hydrocarbon mixture by individual

microorganisms which actually grow on other hydrocarbons within the mixture, a phenomenon called *cometabolism* [Leahy and Colwell, 1990]. The generated by-products of the cometabolic process can often be utilized by other microorganisms and therefore be further degraded [Riser-Roberts, 1992]. Mineral oil products with its multitude of potential primary substrates provide an excellent chemical environment in which cometabolism can occur [Atlas, 1981; Leahy and Colwell, 1990]. Evidence for cometabolism of nondegradable hydrocarbons was provided by Rontani et al. [1985] who reported degradation of asphaltenes in mixed bacterial cultures to be dependent upon the presence of n-alkanes ($12 \geq n \leq 18$). Since cometabolism rarely results in the complete mineralization of contaminants, it may allow accumulation of transformation products, which may be more or less toxic than the original substances [Riser-Roberts, 1992]. Although the autochthonous microorganisms in soils show a broad capability to degrade a variety of contaminants, they are, in some cases, extracted from the contaminated soil, cultivated in a reactor, and re-introduced into the soil as a start-up culture. In other cases, site-independent cultures with known degradation capabilities are introduced in the soil as *superbugs*, but their usefulness is questionable because they are not adapted to the local environmental soil conditions [Thalmann and Küng, 1997]. Furthermore, the acceptance in the public decreases if site-independent organisms are used during the bioremediation processes [Thalmann and Küng, 1997].

1.4 Regulatory standards for soil cleanup

Residual contaminants that are remaining in soils are of considerable concern to many regulatory agencies. In particular for complex mixtures such as mineral oil products, the development of soil cleanup guidelines are causing difficulties due to the lack of compositional as well as toxicological information about the corresponding constituents. Since residual contaminants remain in the soil after the bioremediation has been completed, the question arises whether or not these soils could be reused. In spite of these difficulties, target cleanup guidelines or standards were introduced in many States in the U.S. for a surrogate parameter, the so-called Total Petroleum

Hydrocarbons (TPH) [Tamlyn et al., 1993]. Generally, these numerical guidelines are not risk-based [Millner et al., 1992] and vary considerably from State to State. Soil and groundwater regulations for selected States in the U.S. as well as the corresponding regulations for The Netherlands, Germany, and Switzerland are summarized in Table 1-3. The concentrations required by most of these regulations

Table 1-3 *Regulations for TPH contaminated soil and groundwater for selected States in the U.S. and Europe*

State / Country	Regulations for TPH		References
	in soil [ppm]	in groundwater [ppm]	
United States (cleanup standards)			
Maine	10	0.05	Tamlyn et al., 1993
Pennsylvania	10	< detection limit	"
Oklahoma	50	2	"
Missouri	50-500	5-10	"
Nevada	100	-	"
Kansas	100	-	"
Georgia	100-500	-	"
Washington	200	1	"
Louisiana	< 300	background conc.	"
The Netherlands	50 [1] ; 5'000 [2]	0.05 [1] ; 0.6 [2]	MHSPE, 1994
Germany (for excavated material)	100; 300(500); 1000 [3]	-	LAGA, 1994
drinking water standard		0.01	BGB I, 1986
Switzerland (for excavated material)	50; 300 [3]	2 [4]	BUWAL, 1993 / AltlV [4]
drinking water standard		0.02	FIV, 1995

1) *reference value*
2) *action level*
3) *depending on future landuse*
4) *action level suggested by "Altlastenverordnung" (AltlV) [BUWAL b, in preparation]*

can hardly be reached after the bioremediation processes have been completed. Consequently, the soils cannot be reused but have to be disposed of in landfills, which is unsatisfactory for both, economic as well as ecological reasons.

A major aspect which is not considered by simply comparing the numerical cleanup standards with the TPH concentrations in the soil are the weathering processes of petroleum hydrocarbons in soils. These processes, as discussed earlier and illustrated in Figure 1-3, have a great influence on the chemical composition of the remaining mixture of contaminants. As a result of the bioremediation processes, high molecular components preferably remain in the soil, finally leading to an increase in average molecular weight of the residual contaminants. Compared to the initial contamination, the residual contaminants are more viscous and posses lower overall aqueous solubility [Rosenblatt et al., 1994]. Consequently, completely different mixtures would be compared with each other, if only the TPH would be considered for the determination of cleanup standards for contaminants in soils, and no information about the environmental behavior of the constituents would be taken into consideration. It is more and more accepted that site specific issues such as type of contaminants (e.g. volatile gasoline vs. nonvolatile lubricating oil), size of the spill and distance to the environmental receptors (e.g. the depth to the groundwater) must be considered in order to determine remediation goals [Tamlyn et al., 1993]. The regulatory agencies of some States in the U.S. (e.g. Arkansas, California, Iowa, and others) have recognized these issues and therefore recommend that a risk assessment be performed to develop site-specific remediation goals [Tamlyn et al., 1993].

Decisions concerning cleanup standards in soils should be based on information about the potential emissions and the potential environmental hazards caused by these contaminants. This information could then serve as a basis to conduct a risk assessment for specific contaminants at a particular site.

1.5 Risk assessment

In order to conduct a risk assessment, the U.S. National Academy of Sciences created a *four-stage process* approach which eventually was applied by the U.S. Environmental Protection Agency (U.S. EPA) [La Grega et al., 1994]. This widely recognized approach of the four-stage process include:

- *HAZARD IDENTIFICATION*
- *EXPOSURE ASSESSMENT*
- *TOXICITY ASSESSMENT*
- *RISK CHARACTERIZATION*

The evaluations performed in the course of a risk assessment can then serve as a basis for selecting the appropriate regulatory responses to a potential environmental hazard, also termed as *risk management* [La Grega, 1994]. The following four sections describe each of the four stages as an overview of the state of the art in risk assessment.

1.5.1 Hazard identification

Risk assessment requires a clear understanding of which chemicals are present at what concentrations, and how they could move in the environment from the contaminated site to potential receptors. Since it is common to detect many different chemicals at a contaminated site, a set of selected model compounds, the so called *chemicals of concern*, must be identified which represents indicators for all detected chemicals. This especially is true if mineral oil products are involved. The commonly selected chemicals of concern for fresh mineral oil products are presented in Table 1-4. If altered complex mixtures of petroleum hydrocarbons need to be evaluated (Figure 1-3 C), however, the only information about the variety of unknown organic compounds therein very often is the total amount analyzed, expressed by the TPH.

Table 1-4 *Commonly selected chemicals of concern in different mineral oil products, adapted from ASTM [1995]*

Chemicals of concern	Gasoline	Kerosene	Diesel fuel / Heating oil	Heavy fuel oils
Benzene [1]	X	X	X	
Ethyl-benzene [1]	X	X	X	
Toluene [1]	X	X	X	
Xylenes [1]	X	X	X	
MTBE, TBA, MEK, MIBK,[2]	when suspected	when suspected		
Methanol, Ethanol, Lead	when suspected	when suspected		
(EPA-) PAHs [3]		X	X	X
n-Hexane	X			

[1] *Summarized as BTEX*
[2] *Methyl tertiary-butyl ether, Tertiary-butyl alcohol, Methyl ethyl ketone, Methyl iso-butyl ketone*
[3] *Polycyclic Aromatic Hydrocarbons; commonly referred to the 16 PAHs regulated by the U.S. EPA*

1.5.2 Exposure assessment

The second stage of a risk assessment consists of estimating the exposure to the relevant chemicals by an environmental receptor potentially at risk. It must be analyzed how the contaminants might be released from the site and how they may migrate to a potential receptor. As a result, exposure pathways which are defined by a contaminant source (e.g., landfill), chemical release mechanism (e.g., leaching), transport mechanism (e.g., groundwater flow), exposure point (e.g., drinking water well), environmental receptor (e.g., consumer of drinking water), and exposure route (e.g., ingestion) must exist to result in exposure [La Grega et al., 1994]. Potential exposure pathways of chemicals escaping from contaminated soils are illustrated in Figure 1-4.

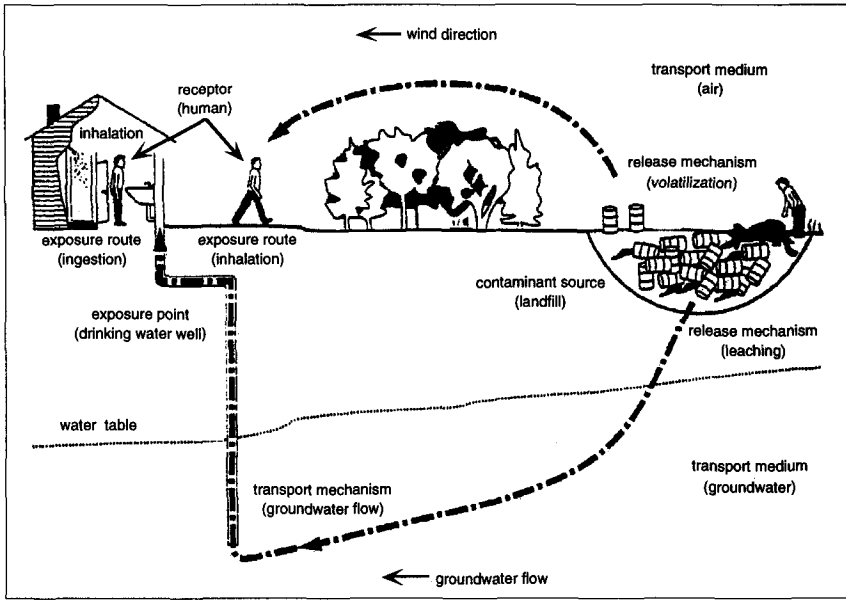

Figure 1-4 *Examples of potential exposure pathways of chemicals from contaminated soils, adopted from La Grega et al. [1994].*

1.5.3 Toxicity assessment

The third stage of a risk assessment provides toxicological data for the selected chemicals of concern or estimates of potential adverse effects obtained from calculations based on physicochemical properties of these chemicals and integrated safety factors [La Grega et al., 1994]. Toxicity is defined as the combination of adverse effects in biological systems which may be caused by chemicals under certain conditions, ranging from minor alterations of normal functions to death [Millner et al., 1992]. The toxicity of an individual chemical is typically established based on dose-response studies that estimate the relationship between different dose levels

and the magnitude of their adverse effects [ASTM, 1995]. The dose-response data subsequently is used to identify a *safe* dose or a toxic level for a particular adverse effect. One approach for assessing the toxicity of mixtures would be the *individual constituent* approach [ASTM, 1995; Verhaar, 1995]. In this approach, the toxicity of the chemicals of concern is separately assessed and, assuming that the toxicities are additive, the toxicity of the mixture is equal to the sum of the individual toxicities, using a hazard index [Millner et al., 1992; ASTM, 1995]. This approach is appreciated for the determination of the toxicity of unweathered mineral oil products since data for the commonly selected chemicals of concern for fresh mineral oil products exist. If the toxicity of altered complex mixtures of petroleum hydrocarbons needs to be evaluated, however, the lack of sufficient compositional and toxicological information is often an impediment to this procedure. Nevertheless, the toxic potential of the TPH constituents from the bioremediated soil investigated in this study was evaluated by using a worst case reference compound.

To evaluate the toxicity potential of chemicals to humans, *Slope Factors* (SF) and *Reference Doses* (RfD) are used for carcinogens and non-carcinogens, respectively. The slope factor is calculated as the 95 percent upper confidence limit of the cancer dose-response curve and is expressed as the inverse of dose (e.g. [$1/mg \cdot kg^{-1} \cdot day^{-1}$]) [La Grega et al., 1994]. Unlike carcinogens, the non-carcinogens exhibit a threshold effect. Below this threshold, the chemicals fail to induce any adverse effects in the receptors [La Grega et al., 1994]. This threshold is defined as RfD which is an estimate of a daily exposure to the human population which are likely to be without appreciable risk of deleterious effects over a lifetime [U.S.EPA, 1996c]. A variety of acute toxicity tests using specific test organisms exist to evaluate the potential adverse effects of chemicals in the environment. Sensitive organisms living in environmental compartments of concern (e.g. water fleas, among many others, in water) are tested. A set of data for selected chemicals in mineral oil products are presented in Table 1-5. While SFs and RfDs are well defined values [U.S.EPA, 1996c], the toxicity data for other organisms than humans vary substantially, in terms of endpoint (e.g. lethality, immobilization), test duration, and test results (as indicated by the data presented for *Daphnia magna* in Table 1-5). However, these tests are

Table 1-5 *Toxicity data for chemical of concern typically found in unweathered mineral oil products*

Chemicals of concern	Human				*Daphnia magna*			
	SF_o [1]	SF_i [2]	RfD_o [3]	RfD_i [4]	EC_{50} [5]	t	LC_{50} [6]	t
	[mg·kg^{-1}·d^{-1}]$^{-1}$	[mg·kg^{-1}·d^{-1}]$^{-1}$	[mg·kg^{-1}·d^{-1}]	[mg·kg^{-1}·d^{-1}]	[mg·L^{-1}]	[h]	[mg·L^{-1}]	[h]
n-Alkanes								
n-hexane			0.06	0.057			4	48[c]
n-decane							18	48[c]
n-docosane							>530	48[c]
BTEX								
benzene	0.029	0.029	0.0017	0.0017	19-38	24[a]	250	24[c]
					57	48[a]	200-400	48[b]
toluene			0.2	0.11	270	24[a]	310	24[c]
					15	48[a]	11-310	48[c]
ethylbenzene			0.1	0.29	2.2	24[a]		
o- xylene			2.0	2.0	1	24[a]	100-1'000	24[b]
m-xylene			2.0	2.0	4.7	24[a]		
p- xylene			2.0	2.0	3.6	24[a]		
U.S.EPA PAHs								
acenaphtene			0.06	0.06	> 280	24[b]		
					3.5-41	48[b]		
acenaphtylene								
anthracene			0.3	0.3			0.04-3	48[b]
benzo-a-anthracene	0.73	0.31						
benzo-b-fluoranthene	0.73	0.31						
benzo-k-fluoranthene	0.073	0.031						
benzo-a-pyrene	7.3	3.1					0.005	96[a]
benzo-g,h,i-perylene								
chrysene	0.0073	0.0031					1.9	2[b]
dibenz-a,h-anthracene	7.3	7.3						
fluoranthene			0.04	0.04			0.004	2[b]
							1'300	24[c]
							320	48[b]
fluorene			0.04	0.04	0.43	48[a]		
indeno-(1,2,3-c,d)-pyrene	0.73	0.31						
naphthalene			0.02	0.00086			> 6.7	2[b]
							17	24[c]
							3.4-16.6	48[b]
phenanthrene					1.1	48[a]	0.45	2[b]
							0.2-1.2	48[b]
pyrene			0.03	0.03			1.3	48[a]

1) *slope factor oral [U.S.EPA, 1996a]*
2) *slope factor inhaled [U.S.EPA, 1996a]*
3) *reference dose oral [U.S.EPA, 1996a]*
4) *reference dose inhaled [U.S.EPA, 1996a]*
5) *effect concentration 50%, from: ([a]) Rippen [1992]; ([b]) Verschueren [1996]; ([c]) LeBlanc [1980]*
6) *lethal concentration 50%, from: ([a]) Rippen [1992]; ([b]) Verschueren [1996]; ([c]) LeBlanc [1980]*

valuable since synergetic effects and effects from unexpected contaminants are detected [Moriarty, 1983].

1.5.4 Risk characterization

The final stage of the four-stage risk assessment compares the effective concentrations from the exposure assessment against the tolerated concentration determined by the toxicity assessment. This approach allows the determination of the relative safety or hazard associated with the anticipated exposures. Since a receptor can potentially be affected by more than just one exposure pathway (e.g. drinking water ingestion as well as ambient air inhalation for a resident as presented in Figure 1-4), the hazard index must be further determined.

1.6 Objectives of this thesis

The aim of this thesis was to evaluate the potential environmental risk caused by the residual contaminants remaining in bioremediated soil, based on a risk assessment. Since information on the mobility and the toxicity of the chemicals of concern in fresh mineral oil products (e.g., PAHs, BTEX) are very well investigated and regulated, the emphasis in this study was put on the multitude of unknown compounds within the residual contaminants. In order to perform a risk assessment, the various processes affecting the residual contaminants in bioremediated soils were investigated in a case study. A complex mixture of bioremediated soils was obtained from a remediation company after the treatment had been completed.

The soil mixture consisted of soils which originated from several different sites that previously had been contaminated mainly with diesel fuels. The soils were mixed together and were subsequently remediated. After the bioremediation had been completed, the soil was spread on a landfill for recultivation purposes from where it was available for this study. This bioremediated soil was named with *Pfyn*, according to the location of its reuse. In Figure 1-5, the time course of the various activities on

this soil is illustrated. A major impediment which had to be dealt with throughout this study is the fact that no corresponding, uncontaminated control could be obtained in order to evaluate the *naturally* occurring background contamination. The TPH concentrations which were determined in this soil therefore were considered as residual contaminants in the soil, although it is reported that TPH concentrations can also be found in uncontaminated samples [Ripper et al., 1993].

The *hazard identification* in this study is performed in Chapter 2. It consisted primarily of an investigation of the physicochemical properties of the residual contaminants in bioremediated soils. The *exposure assessment* which is based on laboratory studies

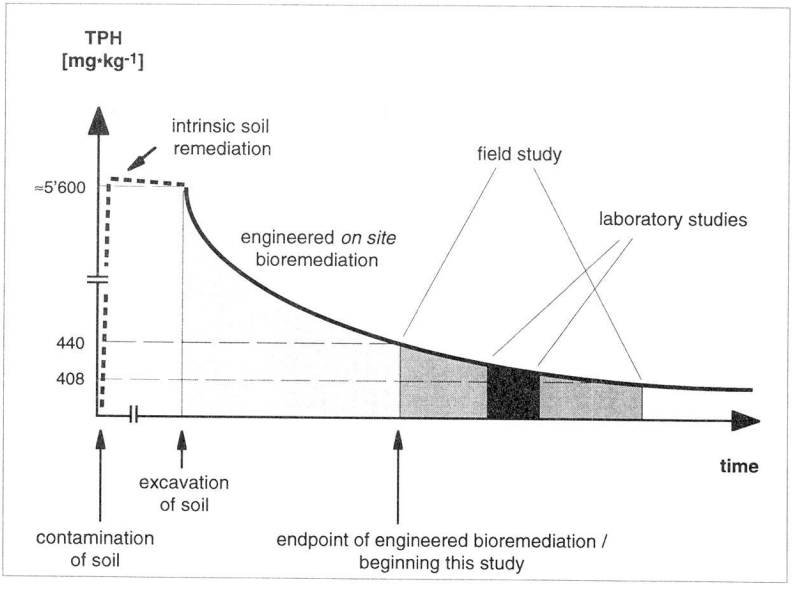

Figure 1-5 *Schematic illustration of the time course of the anthropogenic activities on the soils investigated in this case study*

and field data is presented in Chapter 3. Bioassays described in Chapter 4 as well as toxicity calculations presented in Chapter 5 were conducted to perform a *toxicity assessment.* The *risk characterization* for residual contaminants in bioremediated soils, integrating the results from the exposure as well ass the toxicity assessment, is also presented in Chapter 5. The results obtained from this case study are discussed and the *risk management* is presented in Chapter 6, including a proposal for an alternative approach for regulating TPH contaminants in bioremediated soils as well as mineral oil contaminated soils in general.

2

hazard identification[†]

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PHYSICOCHEMICAL CHARACTERIZATION OF RESIDUAL MINERAL OIL CONTAMINANTS FROM BIOREMEDIATED SOIL

Abstract

After bioremediation of mineral oil contaminated soils, residual contaminants can remain in the soils. In this article, an analytical concept to determine the physicochemical properties of residual contaminants that can be used to assess the environmental fate and potential environmental hazards of these chemicals is presented and discussed. This concept can serve as a tool to characterize unknown compounds within a complex mixture on the basis of basic analytical data. The volatility of the residual contaminants was determined by headspace analyses, but no volatile organic compounds (with a boiling point of < 280°C) were present. Soxhlet extraction using tetrachloromethane was found to be the most efficient method to obtain the Total Solvent Extractable Material (TSEM). Information about the polarity of the contaminants in TSEM was obtained by flash chromatography. It was found that the majority of the residual contaminants are present in the fractions containing nonpolar components, such as branched or cyclic aliphatics and alkylated aromatics. A minor fraction consisted of compounds with polar functional groups, such as long-chain alkanals, alkanols, and alkanoic acids. Information about the octanol water partition coefficient (K_{ow}) of the contaminants within the TSEM was obtained with use of a combination of two independent chromatographic separation steps, reverse-phase-high-performance liquid chromatography, followed by high-resolution gas chromatography. Depending on the compound classes, K_{ow} values varied accordingly but were above 10^6. Considering these K_{ow} values, the mobility of the residual contaminants in bioremediated soils is expected to be very low.

2.1 Introduction

Bioremediation is widely used to treat soils contaminated with organic compounds, particularly when mineral oils are involved [Song et al., 1990]. Compared with thermal and physicochemical treatment technologies, bioremediation has three major advantages: 1) lower remediation costs [Song et al., 1990], 2) the soil as a living system is not destroyed after treatment [Song et al., 1990], and 3) the organic compounds are not just transferred elsewhere but are mainly mineralized [Witt and Nagel, 1997]. However, in most cases, some residual contaminants remain in the soil after bioremediation [Huesemann, 1995]. To quantify and characterize the residual contaminants, they can be solvent extracted and the extract is designated as the Total Solvent Extractable Material (TSEM).

Analyses of TSEM by High-Resolution-Gas-Chromatography (HRGC) usually reveal only few identifiable peaks but a large so-called Unresolved Complex Mixture (UCM) [Oudot et al., 1989; Volkman et al., 1992; Wang et al., 1994; Chaineau et al., 1995]. Although many studies on the biodegradation of crude oil and mineral oil products have been conducted [Walker et al., 1976; Oudot, 1984; Oudot et al., 1989; Gough and Rowland, 1990; Dean-Ross, 1993; Chaineau et al., 1995], the available information about the contaminants remaining in bioremediated soils is very inconsistent. Volkman et al. [1992] described the UCM in bioremediated soils as usually consisting of a multitude of highly branched and cyclic nonpolar compounds. Huesemann [1995] detected polycyclic saturated and aromatic compounds but very few n-alkanes and branched alkanes in bioremediated soils. In contrast, Gough and Rowland [1990] reported that the UCM primarily consists of *monoalkyl substituted T-branched alkanes* and that cyclic products are present only in minor quantities. Chaineau et al. [1995] designated the different fractions as polycyclic alkanes, cycloalkyl aliphatics, polyaromatics, and alkyl-substituted benzenoid structures. Most authors focused mainly on specific compounds, sometimes using uncommon designations, and did not give any information about the total contamination remaining in these soils after treatment. None of the authors mentioned above reported polar compounds within bioremediated soils, although it is well known that during microbiological oxidation of aliphatic and aromatic hydrocarbons, polar

compounds are formed [Singer and Finnerty, 1984; Perry, 1984; Cerniglia, 1984]. Bregnard et al. [1996] mentioned polar transformation products in bioremediated aquifer material and a variety of remaining nonpolar compounds. Langbehn and Steinhart [1995] focused on polar degradation products and showed that during biodegradation in soils contaminated with diesel fuel or lubricants, predominantly alicyclic and branched chain aliphatic organic acids, as well as diacids and aromatic ketones, were formed. However, no data about the physicochemical properties of the variety of unknown contaminants within the TSEM were obtained from the above studies. Consequently, the potential emissions into the environment, as well as the effect on soil fertility and the subsequent risks caused by these contaminants, cannot be assessed. Because of this lack of information, the cleanup levels are set conservatively low and therefore can hardly be reached [Tamlyn, 1993; BUWAL, 1993; LAGA, 1994]. Consequently, the reuse of bioremediated soils is presently strongly restricted by many regulatory agencies, and bioremediated soils must be disposed of in landfills which is unsatisfactory for both economic and ecological reasons. To determine the potential hazard of contaminants remaining in bioremediated soils and to be able to perform a chemical risk assessment, the TSEM needs to be characterized not only in terms of its composition but especially in terms of the fate of these contaminants in the environment. Emphasis was placed on the many unidentified contaminants but not on contaminants of which the corresponding properties are known.

Headspace analyses were conducted to determine the concentration of volatile organic compounds in the bioremediated soil. In addition, flash chromatography [Stephanou and Stratigakis, 1993] and a combination of reverse-phase High-Performance Liquid Chromatography (HPLC) and HRGC [Coates et al., 1985; Davies et al., 1988; Doucette and Andren, 1988; OECD, 1989] was employed to separate the residual contaminants and to obtain data on their polarity and their octanol-water partition coefficient (K_{ow}), respectively.

2.2 Materials and methods

2.2.1 Soil material

A total of 2'200 tons of soil, originating from several different sites contaminated with various mineral oil products (mainly diesel fuel and heating oil), was treated by a remediation company in a prepared bed during one year. Before treatment, the soil was crushed, sieved, and homogenized, and organic structure material (e.g., bark, and straw) was added. During the bioremediation process, the soil material was protected against precipitation by a tent and periodically moistened, homogenized, and aerated by mixing [Witt and Nagel, 1997]. After the treatment was completed, the soil was spread on a landfill to form a layer of approximately one meter. Immediately thereafter, soil samples were taken randomly for investigation. No specific data about the original contamination could be obtained, but during the remediation process, the TSEM decreased from an approximate initial concentration of 10'000 $mg \cdot kg^{-1}$ to a final concentration of about 800 $mg \cdot kg^{-1}$ dried soil. Unfortunately, no information on the changes in composition during the biodegradation process could be obtained.

2.2.2 Chemicals

Tetrachloromethane (99.8 %) was obtained from Scharlau (E.G.T. Chemie, Tägerig, Switzerland) and tetrachloroethene (99.5 %, for IR spectroscopy) from Fluka (Buchs, Switzerland). 1,1,2-trichlorotrifluoroethane (uvasol; 99.9 %) and ethanol (> 99.8 %, p.a.) was purchased from Merck ABS (Dietikon, Switzerland). All other chemicals, including all the reference compounds and silica gel, were obtained either from Fluka (Buchs, Switzerland) or Merck ABS (Dietikon, Switzerland) at the highest purity available. The cellulose filters were obtained from Schleicher & Schuell (Riehen, Switzerland).

2.2.3 Optimization of TSEM extraction from soil

Various methods for extracting organic pollutants from solid materials are proposed by the U.S. EPA [1986] and by DIN in Germany [DIN 38'409, 1981a and b], but no specific method has been adopted so far, e.g. by the Swiss regulators, in combination with cleanup levels. To define the most appropriate method to extract the residual contaminants from bioremediated soil, three different extraction techniques (Soxhlet extraction, cold liquid/sonication extraction, and supercritical fluid extraction) in combination with three halogenated solvents (tetrachloromethane, tetrachloroethene, 1,1,2-trichlorotrifluoroethane) were used. Before extraction, the soil was air dried, crushed (< 5 mm), sieved (< 2 mm) and homogenized. The water content of the soil was below 2 % and for additional drying, 5 g Na_2SO_4 were added to 50 g soil samples and mixed thoroughly before extraction. The extracts were analyzed by infrared spectroscopy (IR) without using further cleanup steps.

Soxhlet extraction : 50 g soil samples were extracted with 200 mL of solvent for 12 hours. The same soil sample was extracted again twice, after replacing the soil extract with fresh solvent.

Cold liquid/sonication extraction : 200 mL of solvent were added to 50 g soil samples in a 500 mL Teflon-lined screw cap flask. After 10 min of sonication (250 W, 33 kHz; Telsonic, Effretikon, Switzerland), the samples were shaken horizontally for 12 hours. After removing the solvent by filtration, fresh solvent was added and the extraction of the same soil sample was repeated twice.

Supercritical fluid extraction : Supercritical fluid extraction (SFE) was performed using an Jasco SFE system (model 100D, Jasco International, Tokyo, Japan). 8 g of contaminated soil sample were placed in a 10 mL extraction vessel and extracted for 30 min using SFE-grade carbon dioxide. 1 mL of tetrachloromethane was used as modifier. The pressure during extraction was 400 atm, and the extraction temperature was set at 150°C. These extraction conditions were defined after the various extraction parameters (temperature, pressure, modifiers and extraction time, respectively) were optimized for the investigated soil. The temperature of the restrictor was approximately 60°C. To collect the extracted components from the soil, 20 mL of tetrachloromethane was used in a collection vial kept at room temperature. Glass beads were used as trap packing material.

The TSEM concentrations were quantified by IR in triplicates. Soxhlet was determined to be the most efficient TSEM extraction method with 2'083 (88), 1'993 (85), and 1'193 (93) $mg \cdot kg^{-1}$ dry weight for tetrachloromethane, tetrachloroethene and 1,1,2-trichlorotrifluoroethane, respectively. The standard deviation is given in parentheses. Furthermore, it was found that approximately 99% of all the extractable compounds could be obtained within the first 12 hours (data not shown). Approximately 25% to 35% lower TSEM concentrations resulted by using cold liquid/sonication extraction [1'449 (102), 1'325 (93) and 905 (128) $mg \cdot kg^{-1}$ dry weight were obtained for the three solvents]. A selected SFE with tetrachloromethane yielded to approximately 60% of the TSEM concentration determined by Soxhlet. Therefore, Soxhlet extraction for 12 hours was used throughout this study to extract the residual contaminants from bioremediated soil. Estimates on the pK_a of alkanols ($pK_a > 14$ [Schwarzenbach et al., 1993]) and alkanoic acids ($0 > pK_a > 6$ [Schwarzenbach et al., 1993]) indicate that part of the alkanoic acids could be present in soils in the deprotonated form. However, preliminary experiments on soil extraction using different reference compounds (n-alkanes, 1-alkanols, and alkanoic acids) demonstrated that all the compound classes of interest could be extracted quantitatively from the soil (data not shown).

2.2.4 Quantification of the TSEM

Quantification by weight : The weight was determined gravimetrically after the solvent was concentrated in a rotary evaporator (< 2 mL) and subsequently evaporated under a gentle stream of N_2 at room temperature until constant weight was reached.

Quantification by IR : The TSEM concentration was determined using an IR spectrometer (Perkin Elmer, 1600 series, Breitenbach, Switzerland) and compared with a Simard standard, which consisted of 37.5 % (w/w) isooctane, 37.5 % (w/w) hexadecane, and 25 % (w/w) benzene [Douglas et al., 1992]. The sum of the adsorption at the wave numbers 2925 cm^{-1} and 2960 cm^{-1} was used for quantification.

Quantification by HRGC/FID : Concentrations were determined using an average response factor for the n-alkanes used as reference compounds. The HRGC analyses were performed on a Fisions HRGC Mega II apparatus (Fisions Instruments, Rodano, Italy), equipped with a Flame Ionization Detector (FID) and a BGB-5 column (30m x 0.32 mm x 0.25 µm) (BGB Analytik AG, Zurich, Switzerland). The column was kept at 80°C for 5 min, then heated to 300°C at a rate of 6°C·min^{-1} and again kept at 300°C for 15 min. A cold on-column injection technique was used and hydrogen was used as the carrier gas.

The three quantification methods used in this study gave very similar results. If the same concentrated soil extract was analyzed gravimetrically, by IR and by HRGC/FID, the concentrations were 17.1 g·L^{-1}, 14.8 g·L^{-1}, and 12.9 g·L^{-1}, respectively. IR and HRGC/FID analyses were used to quantify the TSEM in soils and the corresponding concentrations in the fractionation processes, respectively.

2.2.5 Headspace measurements

Soil samples were collected at a depth of 25 cm and aliquots (2 g) were put in glass vials, which were immediately sealed with Teflon caps. Analyses of the volatile compounds were performed by HRGC, using a Carlo Erba (vega series 5300) apparatus (CE Instruments, Rodano, Italy) equipped with an FID. The glass vials were heated to 80°C for 30 min and 0.5 mL of headspace was directly injected onto a vocol column (60m x 0.32mm x 3µm; Supelco, Buchs, Switzerland). The column was maintained at 40°C for 15 min, followed by heating to 200°C at 5°C·min^{-1} and from 200 to 260°C at 20°C·min^{-1} and maintained at 260°C for 12 min. Hydrogen was used as the carrier gas.

2.2.6 Flash chromatography

Flash chromatography, a separation technique using a silica gel column with different solvent mixtures, was used according to the method described by Stephanou and Stratigakis [1993]. The method was calibrated using reference compounds from each

fraction. Approximately 1 mg TSEM was dissolved in 1 mL of hexane and added on top of 1.5 g silica gel in a glass column (length: 40 mm; diameter: 10 mm). Five individual fractions (aliphatics, PAHs, alkanals and alkanones, alkanols, and alkanoic acids) were collected in cone shaped flasks. After concentrating the solvent in a rotary evaporator (<2 mL) and subsequently evaporating under a gentle stream of N_2, the TSEM was redissolved in 1 mL of tetrachloromethane and the fractions were analyzed by HRGC. A schematic illustration of this analytical method is presented in Figure 2-1.

2.2.7 HPLC analysis

Tetrachloromethane in the TSEM extract was concentrated in a rotary evaporator and subsequently evaporated under a gentle stream of N_2 at room temperature to constant weight. 50 mg of solvent-free TSEM was redissolved in 50 mL ethanol. The solution was heated to 60°C in a water bath for 30 min and sonicated (450 W, 35 kHz; Telsonic, Effretikon, Switzerland). The clear solution was obtained by centrifugation (> 2780 g for 20 min at 20°C) and a recovery of 94 % TSEM in ethanol was achieved by comparing the UCMs in both solvents (tetrachloromethane and ethanol). It is assumed that the differences are due to the reduced solubility of non polar constituents in the polar solvent. However, it is further assumed that the more polar and consequently the more mobile constituents have been quantitatively dissolved in ethanol. 25 µL of TSEM in ethanol were separated into different fractions using an apparatus consisting of a Nucleosil 100-5 C_{18} reversed phase column (Macherey & Nagel, Oensingen, Switzerland), a HPLC pump (600 E, Waters, Milford, MA, USA) and an autosampler (700 satellite WISP, Waters, Milford, MA, USA). The eluting solvent was 100% ethanol with a flow rate of 1 mL·min^{-1} and a pressure of about 175 bar. During 12.5 minutes, 25 fractions, 0.5 mL each, were collected. For analytical reasons, twenty independent separations of TSEM were run, and the corresponding fractions were combined. The volumes of these fractions subsequently were reduced to exactly 1 mL using a Techne-concentrator at 90°C. A schematic illustration of this analytical concept is presented in Figure 2-2.

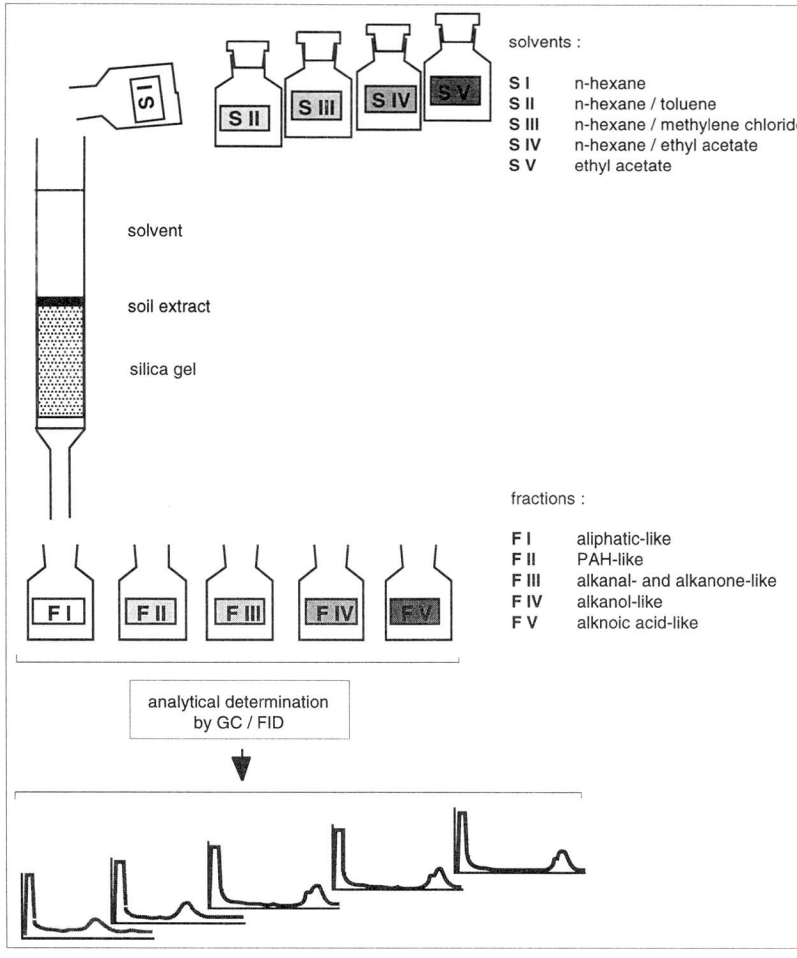

Figure 2-1 *Schematic illustration of flash-chromatography, an analytical separation method of constituents within a complex mixture according to their different polarity [Stephanou and Stratigakis, 1993].*

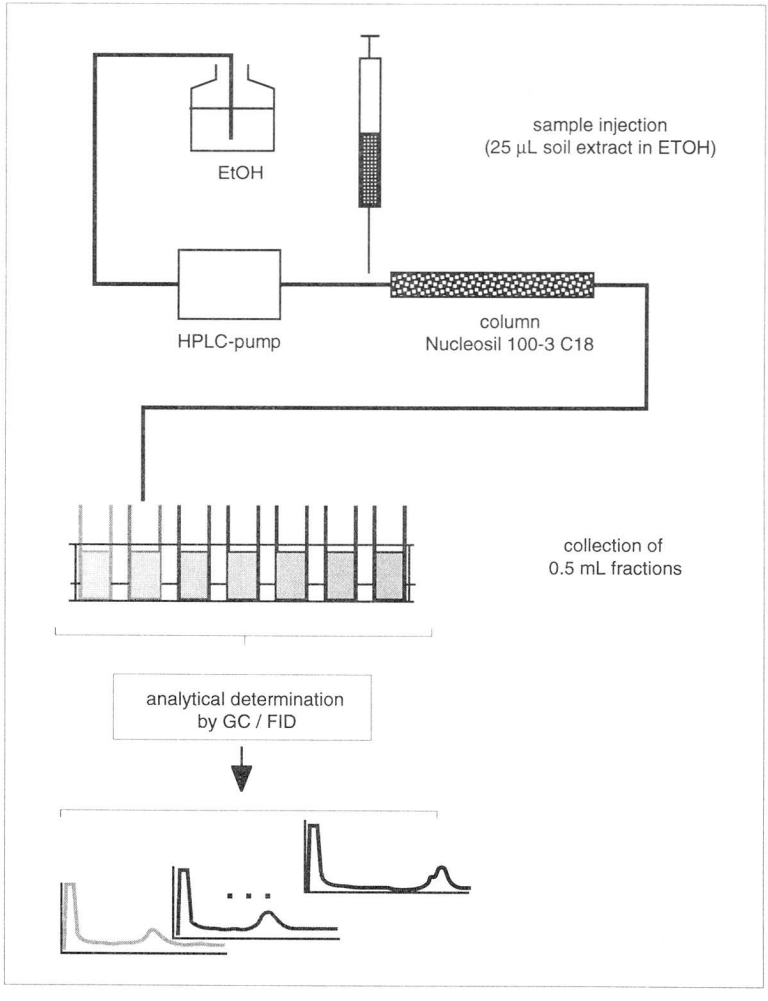

Figure 2-2 *Schematic illustration of the combined HPLC/HRGC separation, an analytical method to separate constituents within a complex mixture according to their different P_{ow} values.*

2.2.8 HRGC-MS analysis

HRGC-MS analyses were carried out on a Hewlett-Packard apparatus (model 5890, Urdorf, Switzerland) with a mass-selective detector and the appropriate data system. The HRGC was equipped with a fused silica capillary column (DB-1;30 m x 0.32 mm x 0.25 µm; J & W Scientific, Koeniz, Switzerland) and helium was used as carrier gas. The electron impact ionization conditions were as follows: ion energy, 70 eV; ion source temperature, 175°C; and mass range m/z, 50-550. The temperature program used for HRGC/FID was also used in this analysis.

2.3 Results and discussion

2.3.1 Volatility of the residual contaminants

A chromatogram of the TSEM from bioremediated soil is presented in Figure 2-3, and shows the UCM at HRGC retention times between 20 and 60 minutes. Assuming that the TSEM consists of n-alkanes, these would correspond to a range between C_{16} and more than C_{35} with a boiling point ranging from 280° to more than 450°C [Verschueren, 1996]. The lack of compounds with retention times < 20 min indicates that no volatile contaminants remained in the bioremediated soil.

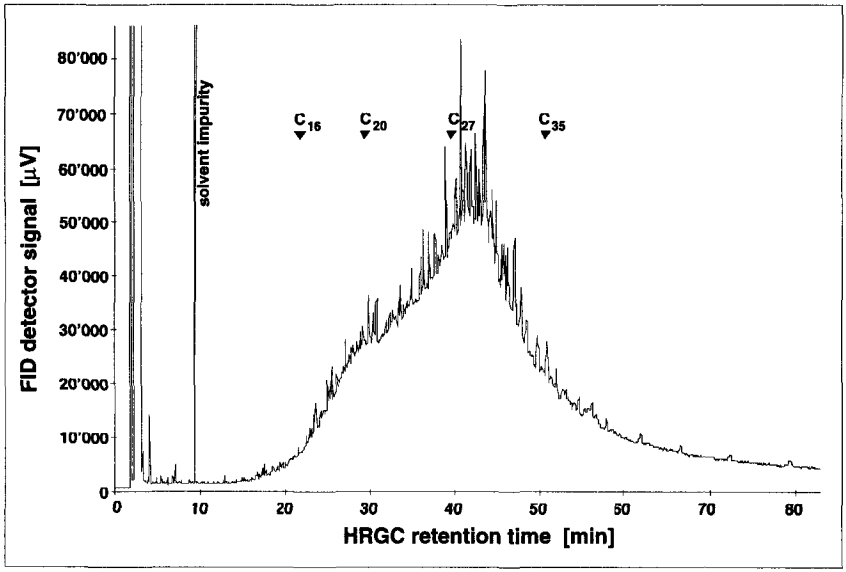

Figure 2-3 *Typical HRGC analysis of the TSEM, dissolved in tetra-chloro-methane. The retention times for selected n-alkanes as reference compounds are marked with an arrow.*

This finding could be confirmed by the results obtained by direct headspace measurements using bioremediated soil, in which the concentrations were below the detection limit of 2 $mg \cdot m^{-3}$ (data not shown). Medium-chain aliphatics (C_{10}-C_{16}), as compared with long-chain aliphatics (>C_{16}), are known to be eliminated faster during the biodegradation process (because of their higher water solubility and their higher bioavailability) and therefore are expected to be completely biodegraded during the remediation process [Oudot, 1984; Kennicutt, 1988]. In addition, as a result of the excavation and homogenization activities, the more volatile compounds are expected to escape from the soil during the bioremediation process. However, because of the initial contaminants in the soil, only low emissions into the atmosphere must be expected and leaching was not causing any emissions because the soil remediation took place in a tent. Therefore, the majority of the eliminated contaminants was due to microbial degradation of the compounds.

Although Chaineau et al. [1995] mentioned that n-alkanes between n-C_{14} and n-C_{27} are rapidly and quite completely removed, it could be demonstrated by HRGC-MS (SIM mode, m/z=85) that some n-alkanes with carbon numbers between C_{17} and C_{27} still remained in the soil but only at very low concentrations (in total less than 0.4 % of the TSEM). It is assumed that these low concentrations remained in the soil because of strongly reduced degradation rates, caused by potential anaerobic microsites, as well as the low bioavailability.

2.3.2 Polarity of the TSEM

Flash chromatography was used to separate the TSEM into five different classes of compounds based on their solubility in the various solvent mixtures and subsequently on the basis of their polarity [Stephanou and Stratigakis, 1993]. Soxhlet extraction with tetrachloromethane is favorable for extracting nonpolar compounds from the soil. However, preliminary studies showed that compounds with polar functional groups, which would be expected as a result of the biotransformation processes, could also be extracted by this method (data not shown). As shown in Figure 2-4, approximately 90 % of the TSEM from bioremediated soil consisted of nonpolar organic

compounds. 77 % were found in the fraction of aliphatics (F_I), and 12 % were found in the fraction of PAH-like compounds (F_{II}).

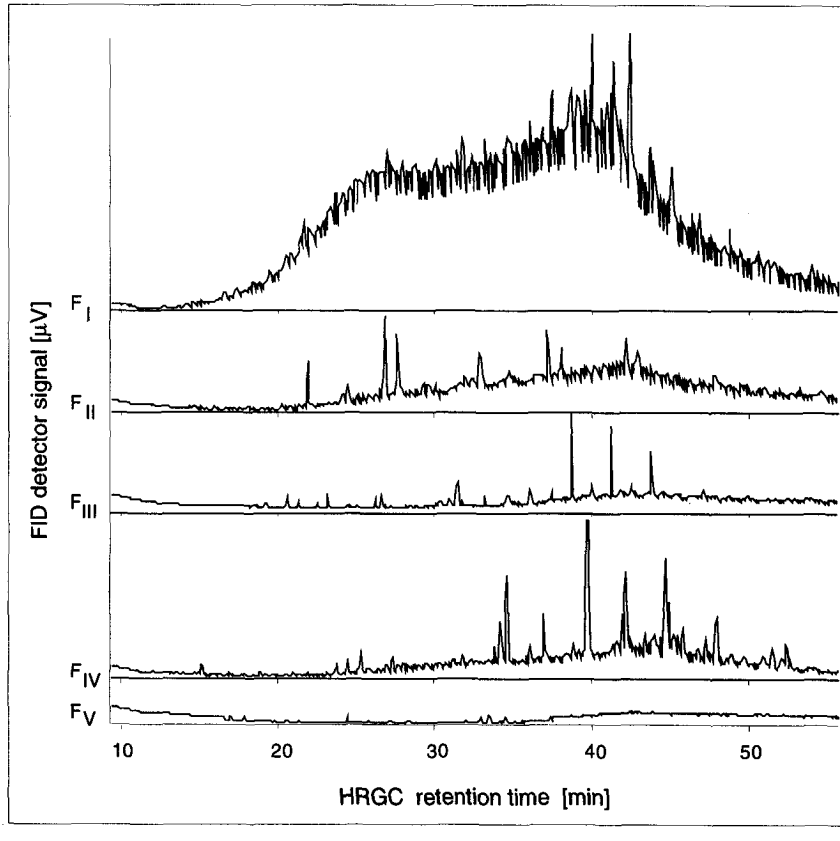

Figure 2-4 *Separation of the TSEM by flash chromatography [Stephanou and Stratigakis, 1993] into fractions predominantly including:*
F_I: aliphatics (77.4 % of TSEM); *F_{II}: PAHs (12.1 %)*
F_{III}: alkanals and alkanones (1.8 %); *F_{IV}: alkanols (8.6 %)*
F_V: alkanoic acids (0.1 %)

Based on the retention times on the HRGC as well as on the assumption that fraction F_I consists of alkanes, the number of carbons for these alkanes range between C_{16} and C_{35}, which is in good agreement with the data obtained from HRGC analysis of the UCM. Some n-alkanes ($C_{16} < n < C_{27}$) as well as isoprenoids (i-C_{19}, and i-C_{20}) were detected and identified by HRGC-MS, but only at very low concentrations (a total of approximately 0.4% of the TSEM). Therefore, the major portion of F_I must be nonpolar compounds other than n-alkanes (i.e., cyclic and/or branched hydrocarbons) that were not identified. In fraction F_{II}, less than 3 % of this fraction and subsequently less than 0.4% of the TSEM correspond to the sixteen PAHs regulated by the U.S.EPA. Phenanthrene, fluoranthene, pyrene, chrysene, and benzo[k]fluoranthene were found in concentrations between 0.2 and 0.4 $mg \cdot kg^{-1}$ soil. Therefore, most of the compounds within F_{II} are supposed to be alkylated PAHs or representatives of other compound classes with very similar polarity.

The remaining 10 % of the TSEM were found in the fractions for compounds with some polar functional groups. Alkanal- and alkanone-like compounds (fraction F_{III}), alkanol-like compounds (fraction F_{IV}), and alkanoic acid-like compounds (fraction F_V) represented portions of 1.8 %, 8.6 %, and 0.1 %, respectively. According to the library search on the HRGC-MS, fraction F_{III} includes alkanals with numbers of carbons ranging between C_{14} and C_{26}. Assuming that fraction F_{IV} would only consist of 1-alkanols, the HRGC retention times indicate that the corresponding number of carbons would range between C_{16} and C_{36}.

2.3.3 K_{ow} range of the TSEM

To obtain information about the K_{ow} values of the compounds within the TSEM, they were separated using a combination of two independent chromatographic methods, HPLC and HRGC.

Selection of reference compounds: The results obtained by flash chromatography showed that the TSEM fraction representing aliphatics was quantitatively the most important one. Therefore, n-alkanes were used as reference compounds to investigate the separation behavior of the hydrophobic compounds. Because the

mobility in soil is expected to be enhanced for compounds with polar functional groups, 1-alkanols and alkanoic acids were additionally used as reference compounds. Although the fraction representing PAH-like compounds is important, no PAHs were used as reference compounds, because their physicochemical properties are known and can be obtained from the literature [Verschueren, 1996].

Separation of reference compounds: To calibrate the HPLC and HRGC methods, the reference compounds from the different homologue series were separated independently. The separation behavior of the reference compounds during the two chromatographic procedures is presented in Figure 2-5. In addition, literature data on boiling temperatures and K_{ow} values for the corresponding compound classes are also presented in Figure 2-5. The HRGC retention times (Figure 2-5A) and consequently the boiling points (Figure 2-5B) for the reference compounds depend not only on the number of carbons but also on the presence of functional groups. Compounds with polar functional groups (1-alkanols, and alkanoic acids) show significantly higher HRGC retention times than the corresponding n-alkanes. The information in Figure 2-5 A and B can be combined as indicated by the arrows. If the retention time is determined from a HRGC chromatogram for an unknown compound, the number of carbon atoms (N_c) of this component can be determined graphically using the corresponding homologue series in Figure 2-5 A (in this example, n-octadecane was used). Subsequently, the boiling temperature can be obtained from Figure 2-5 B, leading to approximately 320°C. The value found in the literature for n-octadecane is 317°C [Verschueren, 1996].

As indicated in Figure 2-5 D, compounds with polar functional groups have K_{ow} values that are about two orders of magnitude lower than those of the corresponding n-alkanes. Therefore, compounds with polar functional groups are eluting within earlier HPLC fractions (Figure 2-5 C). Within homologue series, low-molecular-compounds possess lower K_{ow} values and consequently lower HPLC retention times than high-molecular-compounds. In the guideline of the Organization for Economic Cooperation and Development [OECD, 1989], a linear correlation between the log K_{ow} and HPLC retention times is suggested for any compound within a log K_{ow} range of 0 to 6. It could be demonstrated in this study, however, that this correlation is not

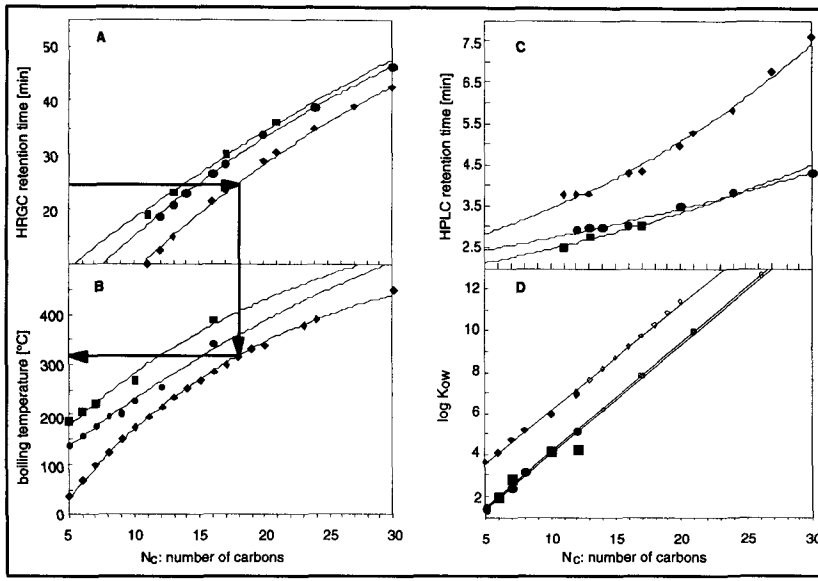

Figure 2-5 *Characteristics of the reference compounds n-alkanes (◆), 1-alkanols (●) and alkanoic acids (■) as a function of their chain lengths (N_c):*

***A**: experimentally determined HRGC retention times for the different homologue series.*

***B**: boiling temperatures for selected reference compounds obtained from the literature [Verschueren, 1996]*

***C**: experimentally determined HPLC retention times for the different homologue series*

***D**: log K_{ow} values for selected reference compounds; filled: obtained from the literature [Verschueren, 1996]; open: estimated according to Hansch and Leo [1979]*

valid in general but only for compounds within the same homologue series, presumably because the log K_{ow} value for the compounds investigated is beyond the suggested log K_{ow} range. Therefore, each compound class had to be investigated individually and no K_{ow} predictions can be made for unknown compound classes. To determine log K_{ow} values from HPLC retention times for unknown components, Figures 2-5 C and D can be combined and the corresponding values obtained graphically, as described earlier for the determination of the boiling temperature of n-octadecane.

Separation of the TSEM compounds: The fate of the TSEM during this HPLC/HRGC separation procedure was compared with that of known reference compounds. If two compounds behave similarly during both chromatographic separation steps, there is strong evidence that their mobility in the environment is also similar. As presented by the selection of HRGC chromatograms of single HPLC fractions in Figure 2-6, the TSEM could be further separated. The regions where the corresponding reference compounds are expected in each of the HPLC fractions are indicated in Figure 2-6 as well.

According to the regions representing n-alkanes, the components eluting in fractions F_9 to F_{21} as UCM with a narrow range of boiling temperatures must be nonpolar compounds with physicochemical properties similar to those of n-alkanes. According to the HRGC retention times, their corresponding chain length must be above C_{16}. These results could additionally be confirmed by separating the TSEM, which was previously spiked with nonpolar reference compounds. It was indicated that all reference compounds eluted within the defined regions (data not shown). In fractions F_9 to F_{13}, a second but smaller UCM is also present at slightly higher temperatures. According to the physicochemical properties of low-molecular-aliphatics, cycloalkanes have higher boiling temperatures and lower log K_{ow} values than the corresponding n-alkanes [Verschueren, 1996; Mott, 1995]. Therefore, it is assumed that this second UCM could represent cycloalkanes. The lowest estimated log K_{ow} range for the nonpolar UCM compounds in fraction F_9 and subsequently for all the nonpolar UCM compounds in the following fractions is above eight. Because

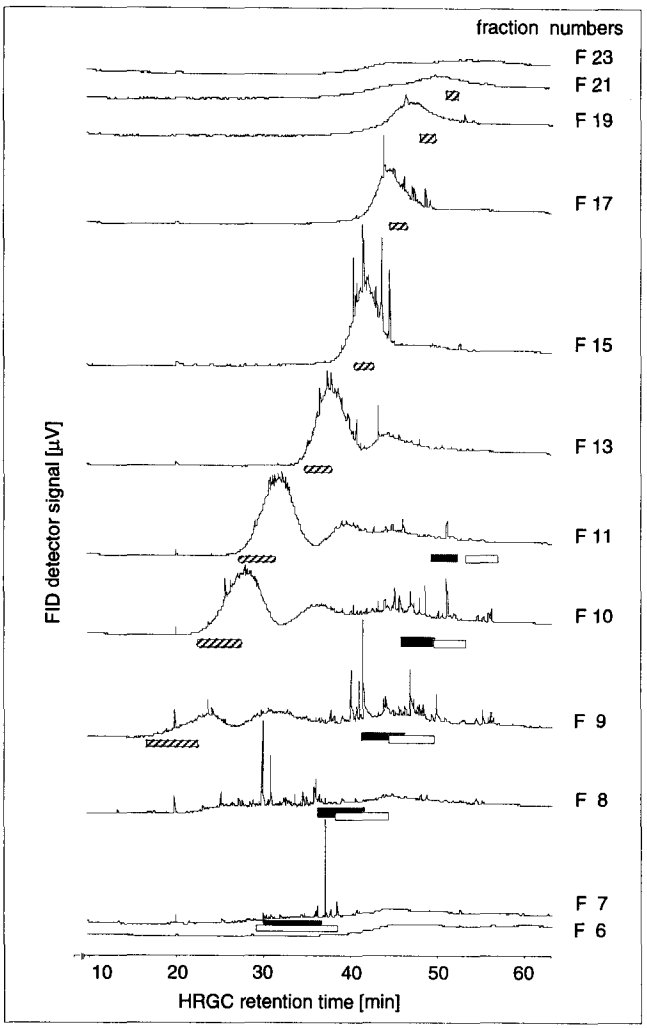

Figure 2-6 *Selected HRGC-chromatograms of single fractions obtained after separating the TSEM by HPLC. The regions representing the different reference compounds are: ▨ n-alkanes; ▬ 1-alkanols ▭ alkanoic acids. Each fraction is representing 0.5 min of HPLC retention time, from fraction F_6 (2.5-3.0 min) to fraction F_{23} (11.0-11.5 min).*

calculated log K_{ow} values above eight can be associated with serious error, these data should be treated with extreme caution [Mackay et al., 1992]. However, these high log K_{ow} values indicate, that the hydrophobicity is large and subsequently the mobility of the residual compounds from the soil is expected to be extremely low. As mentioned earlier, special interest was placed on the content of the earlier fractions because these compounds must have lower K_{ow} values and consequently a higher mobility in soils. The distinct signals appearing over a wide range of higher HRGC retention times in the earlier fractions (F_7-F_{10}) are expected to be compounds with polar functional groups because they match with the regions representing 1-alkanols and alkanoic acids (Figure 2-6). None of these compounds could be identified by HRGC-MS. However, according to Hansch and Leo [1979], the lowest log K_{ow} range is estimated to be 6.2 and 7.8 for 1-alkanols and alkanoic acids, respectively. The resulting log K_{ow} values for the TSEM compounds therefore would range between 6.2 and more than 8. Furthermore, it is indicated that even for TSEM contaminants with polar functional groups, the mobility from the soil is expected to be extremely low. The total of all the contaminants with polar functional groups within the fractions F_7 to F_{10} represent approximately 10 % of the TSEM, indicating that the concentration of contaminants with log K_{ow} values less than 8 is very low in this bioremediated soil.

The results obtained from this study are summarized in Table 2-1. The physico-chemical properties of the residual contaminants in bioremediated soils can serve as a basis to estimate the environmental fate and the potential environmental hazards they may pose. According to the vapor pressure of the compounds within the TSEM, the volatility is very low; consequently, the emissions into air are expected to be negligible. The K_{ow} of the compounds within the TSEM was shown to be above 10^6; subsequently, the mobility in the soilfor all the residual contaminants is negligible. From these findings, it is expected that only low emissions from bioremediated soils into the environment might occur. This prediction, however, must be experimentally examined because colloid-facilitated transport could affect the mobility of compounds with high K_{ow} values. In the following chapter, emphasis was put on the experimental assessment of the movement and fate of the TSEM under laboratory and field

conditions. Furthermore, the analytical concept and results presented in this chapter will be used to estimate future emissions from bioremediated soils by performing a mass-flow analysis.

Table 2-1 *Overview of the characteristics of the soxhlet extracted TSEM from bioremediated soil*

			Physicochemical characterization		
Compound classes, based on Figure 2-4		[%] [1]	log K_{ow} [2] [-]	b.p. [2] [°C]	P°(L) [3] [atm]
Aliphatic-like compounds	(F_I)	77.4	> 8	> 280	< 8.5×10^{-6}
PAH-like compounds	(F_{II})				
- total		12.1	n.d. [5]	> 300	
- EPA-PAHs only		0.4	> 4.5 [4]	> 340 [4]	< 5.9×10^{-7} [4]
Alkanal- & alkanone-like compounds	(F_{III})	1.8	n.d. [5]	n.d. [5]	
Alkanol-like compounds	(F_{IV})	8.6	> 6.2	> 350	< 2.7×10^{-9}
Alkanoic acid-like compounds	(F_V)	0.1	> 7.8	> 420	< 5.7×10^{-10}

[1] *determined by flash chromatography / HRGC-MS*

[2] *data determined from Figure 2-5*

[3] *vapor pressure of the liquid compound at 20°C, estimated from b.p. according to Schwarzenbach et al. [1993]*

[4] *literature data for phenanthrene [Verschueren, 1996]*

[5] *not determined*

3

exposure assessment‡

‡ accepted for publication by *Environmental Toxicology and Chemistry* under the title:

MOVEMENT AND FATE OF RESIDUAL MINERAL OIL CONTAMINANTS IN BIOREMEDIATED SOIL

Abstract

After completion of the bioremediation of soils contaminated with mineral oil products, residual contaminants remain in the soil matrix. Since only limited information is available about the movement and fate of these residual contaminants, the potential environmental hazards are difficult to assess. The aim of this study was: a) to identify and quantify the relevant transport and transformation processes of the residual contaminants in bioremediated soil such as volatilization, leaching and biodegradation and b) to conduct a mass-flow analysis for the Total Solvent Extractable Material (TSEM) in bioremediated soil for the first year after application as top soil, based on experimental data as well as model calculations. The results indicate that the major portion of the residual contaminants (93 % of the initial TSEM after one year of the application as top soil) will remain in the bioremediated soil for a long time period. The 7 % of the initial TSEM which were lost during this time period could be further divided into different processes: 98 % of the total losses were due to transformation processes, a combination of biodegradation and aging effects. Losses due to volatilization of the contaminants into the atmosphere as well as due to plant uptake were estimated to be negligible (< 0.001 % of the losses). Leaching, although identified as the major transport process, accounted for only 1.7 % of the losses.

3.1 Introduction

Numerous studies and reviews on the biodegradation of crude oil and mineral oil products in soils have been published [Kennicutt, 1988; Song and Bartha, 1990; Song et al., 1990; Langbehn and Steinhart, 1995; Riis et al., 1995; Geerdink et al., 1996]. However, very little is known about the movement and fate of the 10 to 30 % of the initial organic contaminants usually remaining after bioremediation [Miethe et al., 1994]. Microcosm studies revealed that residual contaminants from weathered diesel fuel in aquifer material can be further biodegraded under aerobic and anaerobic conditions [Bregnard et al., 1996]. When the Total Solvent Extractable Material (TSEM) from bioremediated soils is investigated [Oudot et al., 1989; Volkman et al., 1992; Wang et al., 1994; Chaineau et al., 1995], a so called Unresolved Complex Mixture (UCM) can be detected in High Resolution Gas Chromatographic analysis (HRGC), and individual component classes therein were characterized [Walker et al., 1976; Oudot, 1984; Gough and Rowland, 1990; Dean-Ross, 1993]. However, current environmental legislation in many countries is based on the TSEM and the Total Petroleum Hydrocarbon (TPH) concentrations in the soils [Tamlyn et al., 1993; VSBo, 1993; LAGA, 1994]. Due to the lack of knowledge regarding the movement and fate of the residual contaminants and the potential risk associated, excavated bioremediated soil in Switzerland is classified as waste [USGrev, 1997] and can, therefore, presently not be reused but has to be disposed of in landfills. This solution, however, is unsatisfactory for both economic and ecological reasons.

In the previous chapter, the TSEM extracted from bioremediated soil was characterized and the range of physicochemical properties of its constituents have been determined [Chapter 2; Angehrn et al., 1998]. It was found that the octanol/water partition coefficients (K_{ow}) of the constituents were above 10^6, indicating that the mobility of the residual contaminants in bioremediated soils can be assumed to be very low. However, as a basis for a more detailed risk assessment for residual contaminants from bioremediated soils, their movement

and fate in the environment was investigated in a case study. Volatilization and leaching, the most important transport processes for residual contaminants from bioremediated soils [Bossart and Bartha, 1984] were studied. Since abiotic oxidative transformation processes (chemical/photochemical degradation) of hydrocarbons in subsurface environments are of little significance [McGill et al., 1981; Bossart and Bartha, 1984], further biodegradation of these contaminants was the only transformation process investigated. A field study as well as laboratory studies were conducted and, based on these experimental data as well as on model calculations, the potential emissions were estimated by a mass-flow analysis for the first year after applying the bioremediated soil as top soil.

3.2 Material and methods

3.2.1 Soil preparation

Soil contamination

Bioremediated soil consisting of a mixture of soils originating from several different sites contaminated mainly with diesel fuel was obtained from a remediation company after the treatment had been completed. During this remediation process, the TSEM concentrations had been reduced from approximately 10'000 $mg \cdot kg^{-1}$ to a final concentration of 780 $mg \cdot kg^{-1}$ and a final TPH concentration of 430 $mg \cdot kg^{-1}$. The concentrations refer to the dry weight of the fine grained material (sieved < 2 mm) in the soil, referred to as C^s_{TSEM} and C^s_{TPH}, respectively. For leaching and biodegradation studies, the bioremediated soil had to be crushed (< 5 mm) before sieving (< 2 mm). The TSEM and TPH concentrations are expressed for the dry weight of the crushed soil and are referred to as C^{cs}_{TSEM} and C^{cs}_{TPH}. In a given soil sample, C^{cs} is smaller (approximately 40 %) than C^s because the total material and not just the fine grained material is analyzed.

Spiking the soil with TSEM

For leaching and biodegradation experiments, bioremediated soil samples and quartz sand were additionally spiked with residual contaminants which had previously been soxhlet extracted from the bioremediated soil [Chapter 2; Angehrn et al., 1998]. To apply these compounds homogeneously onto the soil surface, it was necessary to dissolve these chemicals in a volatile solvent. Approximately 10 mL of 1,1,2-trichlorotrifluoroethane was used for this procedure. To ensure equal distribution of the contaminants throughout the amended soils, the solution was sprayed onto the soil in three portions with thorough mixing of the soil after each application. The solvent was allowed to evaporate by mixing the soil periodically.

3.2.2 Experimental setup

Field study

Bioremediated soil was spread on top of a landfill for recultivation purposes to form a layer of one meter. On an area of approximately 600 m^2, the natural attenuation of residual contaminants in the bioremediated soil was investigated over a time period of 2.5 years. A schematic illustration of this field site is presented in Figure 3-1. A randomized block design was used to assess four different types of agricultural treatments: unplowed, covered with grass (*Lolium perenne*); unplowed, covered with clover (*Trifolium pratense*); plowed (no vegetation) as well as untreated controls (unplowed, no vegetation). Each type of treatment was applied to three plots covering an area of 7m·7m each. The three plowed plots were treated to a depth of approximately 25 cm every second month and the vegetation on the six plots with plant covers (grass or clover) was cut four times a year and the litter was removed. Periodically, the soil was sampled by taking 20 subsamples per plot to a depth of 50 cm using a square grid sampling design [Ferguson, 1992]. Each

subsample was divided into an upper horizon (0-25 cm) and a lower horizon (25-50 cm). The subsamples of one plot from each horizon were then thoroughly mixed to form one composite sample (Figure 3-1). Thus, two composite samples were obtained from each plot which were further prepared for analysis.

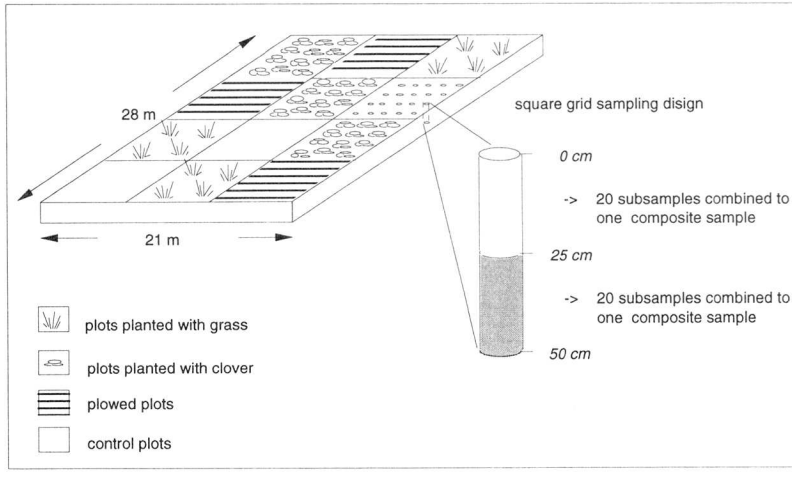

Figure 3-1 *Schematic illustration of the setup as well as the sampling strategy used in the field study*

Laboratory experiments to evaluate the biodegradation of residual contaminants in the soil

In order to investigate the rate of further biodegradation of the residual contaminants in the bioremediated soil, a laboratory degradation experiment was conducted for 12 weeks at a temperature of 22°C ± 2°C. Two soil batches were investigated in this study: bioremediated soil with and without the addition of residual contaminants were used with initial concentrations of C^{cs}_{TSEM}=440 mg·kg^{-1}; C^{cs}_{TPH}=229 mg·kg^{-1} and C^{cs}_{TSEM}=1250 mg·kg^{-1}; C^{cs}_{TPH}=710 mg·kg^{-1}, respectively.

Nutrients (NH_4Cl, K_2HPO_4) were added to both soil batches according to [Klein, 1992] and subsequently, 1300 g of each soil were placed in 3 L glass vessels. The vessels were aerated with CO_2-free, synthetic air which previously had been scrubbed from traces of hydrocarbons and CO_2 by passing through an active carbon cartridge, 2 M KOH-solution and water, respectively. The soil batches were homogenized by rotating the vessels twice a day. The CO_2 produced was continuously absorbed in 2 M KOH, and the concentrations were determined every second week with a TOC-analyzer (Shimadzu; TOC-5050, Burkhard Instruments, Zurich, Switzerland). In addition, 50 g of soil were sampled every second week and analyzed for TSEM and TPH concentrations.

Determination of the volatilization rate of residual contaminants

Soil samples were collected at a depth of 25 cm after the soil had been spread on the landfill and aliquots (2 g) were put in glass vials, which were immediately sealed with Teflon caps. The headspace in the glass vials was analyzed by HRGC/FID as described below.

Leaching experiments in soil columns

660 g and 1000 g of bioremediated soil and spiked quartz sand, respectively, were filled into glass columns (inner diameter: 5 cm; length: 45 cm) which contained a 5 cm filter layer of cleaned quartz sand (0.3-0.9 mm). The experimental setup of the columns is presented in Figure 3-2 A. The columns were watered with deionized water at a rate of 25.5 $mL \cdot cm^{-2} \cdot wk^{-1}$, simulating an annual precipitation of 1400 $L \cdot m^{-2} \cdot yr^{-1}$ within a time period of 5 weeks. Data for the different experimental setups are summarized in Table 3-1. The leachates were collected in 1L glass bottles and analyzed weekly. To prevent microbial growth in the bottle, a pH <2 was achieved by adding 2 mL HCl conc..

Table 3-1 *Experimental data for the different leaching experiments using bioremediated soil and spiked quartz sand*

Experimental parameters	Dimension	Column A [1]	Column B [2]	Lysimeter [1]
Experimental setup				
bioremediated soil	g	660		145'000
quartz sand	g		1'000	
surface area	cm^2	19.6	19.6	2'827
diameter	cm	5	5	60
watering rate	$mL \cdot cm^{-2} \cdot wk^{-1}$	25.5	25.5	11.5
time to simulate annual precipitation	wk	5	5	12
test duration	wk	11	11	24
simulated time period	months	25	25	24
Initial concentrations in soil / quartz sand				
- C^{cs}_{TSEM}	$mg \cdot kg^{-1}$	432	473	445
- C^{cs}_{TPH}	$mg \cdot kg^{-1}$	295	312	299
Initial amount in soil / quartz sand				
- TSEM	mg	285	473	64'525
- TPH	mg	195	312	43'355
DOC concentration in the leachate				
- DOC (day 0)	$mg \cdot L^{-1}$	13.4	5.3	19.0
- DOC (day 84)	$mg \cdot L^{-1}$	13.2	1.4	
- DOC (day 190)	$mg \cdot L^{-1}$			9.1

[1] *filled with bioremediated soil*

[2] *filled with quartz sand which was previously spiked with residual contaminants from the bioremediated soil*

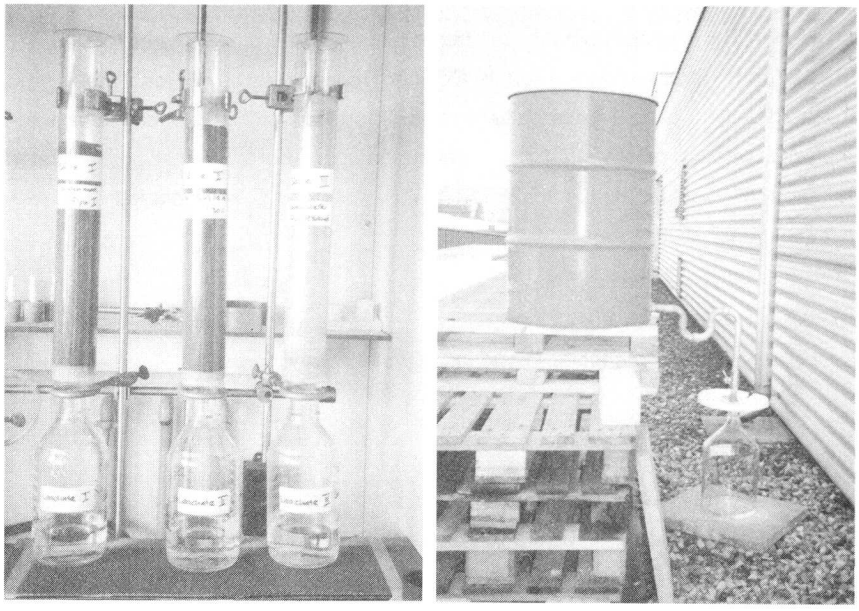

Figure 3-2 *Photographs of the leaching experiments conducted in this study: medium scale columns (a) and a large scale lysimeter (b)*

Leaching experiments in a lysimeter

A leaching experiment was conducted in an outdoor lysimeter (Figure 3-2 B). A barrel with a diameter of 60 cm was equipped with a 15 cm filter layer of cleaned quartz sand (0.3-0.9 mm) and filled with 145 kg of bioremediated soil. This size allowed a desired scale up of the laboratory experiments under ambient outdoor climate conditions. A roof prevented natural precipitation from reaching the lysimeter. Instead, the soil was watered manually at 11.5 $mL \cdot cm^{-2} \cdot wk^{-1}$, a rate simulating an average local precipitation of 1400 $L \cdot m^{-2} \cdot yr^{-1}$ within three months. This six month experiment was conducted during the summer season from late April

to October. The average monthly soil temperature at 10 cm depth were 14, 16, 23, 18, 13 and 12°C for May through October, respectively. The leachates were collected daily in a 10 L glass bottle and analyzed periodically. All leachates were analyzed without employing further cleanup steps. Therefore, colloid facilitated transport of residual contaminants from this soil would be included in these investigations.

Evaluation of biodegradation of residual contaminants in water

The potential biodegradability of residual contaminants in liquid medium was investigated by using the CEC degradation test, a standardized test which originally was developed to determine the biodegradability of engine oils in water [CEC, 1995]. In this test, 150 µg of the residual contaminants, dissolved in 1µL 1,1,2-trichlorotrifluoroethane, were applied to 150 mL liquid medium. Secondary effluent of a municipal wastewater treatment plant was used as inoculum. The biodegradability of the residual contaminants was monitored over an incubation time of 21 days.

3.2.3 Chemical analyses

Residual contaminants in soil air

Soil samples were taken from the subsurface in the field and placed into glass vials which were sealed with Teflon caps immediately thereafter. The glass vials with two grams of soil were heated to 80°C for 30 min. Analyses of the volatile compounds present in the headspace were performed by HRGC, using a Carlo Erba (vega series 5300) apparatus (CE Instruments, Rodano, Italy) equipped with an FID. 0.5 mL of the headspace was directly injected onto a vocol column (60m x 0.32mm x 3µm; Supelco, Buchs, Switzerland). The column was maintained at 40°C for 15

min, followed by heating to 200°C at 5°C·min^{-1} and from 200 to 260°C at 20°C·min^{-1} and maintained at 260°C for 12 min. Hydrogen was used as the carrier gas.

Determination of TSEM and TPH in soil samples

The soil samples were air dried, ground, sieved (<2 mm) and thoroughly mixed with anhydrous Na_2SO_4 (to absorb residual moisture) prior to the soxhlet extraction using tetrachloromethane [Chapter 2; Angehrn et al., 1998]. Quantification of the TSEM concentrations was performed by infrared spectroscopy (IR) (Perkin Elmer, 1600 series, Breitenbach, Switzerland), using a Simard standard [Douglas et al., 1992]. The TPH concentrations were obtained after an alumina column cleanup [U.S.EPA, 1989] to remove polar compounds and subsequently quantified by IR as described in Chapter 2.

Determination of TSEM and TPH in water samples

After acidifying the water samples using HCl conc. (pH <2) and after adding NaCl (5 g per 500 mL of water), the TSEM were extracted with 25 mL tetrachloromethane by shaking for one hour. The solvent phase was separated from the water phase in a separation funnel, dried with Na_2SO_4 and subsequently filtrated through glass wool. The TSEM concentration and subsequently the TPH concentration in the solvent was determined by IR as described above. The quantitation limit of TSEM and TPH in water samples was 0.01 mg·L^{-1}.

Dissolved Organic Carbon (DOC) in water samples

5 mL of a water sample were filtrated (0.45 µm, cellulose membrane, Millipore, Volketswil, Switzerland), 100 µL 2M HCl were added and the sample was analyzed by a TOC-analyzer (Shimadzu; TOC-5050, Burkhard Instruments, Zurich, Switzerland). The quantitation limit was 1 mg C·L^{-1}.

3.3 Results and discussion

3.3.1 Natural attenuation of residual contaminants in soil under field conditions

The results of the field study are presented in Figure 3-3 for the four different types of agricultural treatments. It is evident in all cases that the C^s_{TSEM} decreased only slightly during the investigation period, with an average rate of approximately 55 $mg \cdot kg^{-1} \cdot yr^{-1}$. Therefore, the majority of the residual contaminants, an average of 93 % and 82 % for one and for 2.5 years, respectively, remained in the soil. No differences in the decrease of C^s_{TSEM} and C^s_{TPH} could be observed during the investigations with vegetated and unvegetated plots (Figure 3-3). The biodegradation rate of the residual contaminants was not affected by the presence of plants although several studies suggest that elevated microbial activity is found in vegetated contaminated soils (with diesel fuel and polycyclic aromatic hydrocarbons) as compared to soils without plants [Aprill and Sims, 1990; Shimp et al., 1993; Lee and Banks, 1993; Radwan et al., 1995; Günther et al., 1996; Reilley et al., 1996]. As demonstrated in Chapter 2, the K_{ow} of the residual contaminants is very high [Angehrn et al., 1998] and the water solubility is, therefore, very low. Consequently, the bioavailability of these components is highly reduced.

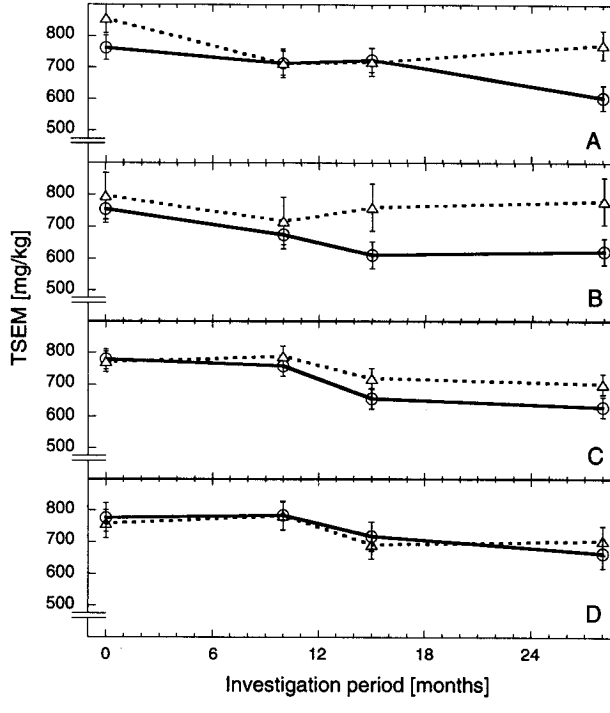

Figure 3-3 *Time course of the TSEM concentrations in the different plots during the 2.5 years of field observations in: A: control plots; B: plowed plots; C: plots planted with clover; D: plots planted with grass. The solid and dashed lines are representing the samples from 0-25 cm and 25-50 cm, respectively and the standard deviation is expressed with error bars.*

3.3.2 Biodegradation of residual contaminants in soil

The potential for further biodegradation of residual contaminants in bioremediated soils was elucidated in laboratory experiments under optimized conditions with respect to nutrients, oxygen supply, and temperature. Bioremediated soil which was additionally spiked with residual contaminants as well as bioremediated soil itself were incubated for twelve weeks. The C^{cs}_{TSEM} and C^{cs}_{TPH} as well as the cumulative CO_2-production are presented in Figure 3-4 as a function of the incubation time. The C^{cs}_{TSEM} and C^{cs}_{TPH} decreased from 1250 mg·kg^{-1} to 990 mg·kg^{-1} and from 720 mg·kg^{-1} to 590 mg·kg^{-1}, respectively, and as a consequence of the addition of residual contaminants to the bioremediated soil (Figure 3-4A), the CO_2-production increased significantly compared to that in Figure 3-4B. The amount of CO_2 produced with an initial rate of 17 mg CO_2·kg^{-1}·wk^{-1} and 105 mg CO_2·kg^{-1}·wk^{-1} in the bioremediated soil and the spiked soil, respectively, corresponded to a mineralization of 85 % of the eliminated contaminants. Therefore, it can be concluded that the disappearance of these contaminants must be due to biological degradation. Approximately 20 % of the initial concentrations in the spiked soil were lost during the investigated period (Figure 3-4A). As presented in Figure 3-4B, the decrease of C^{cs}_{TSEM} was slower in the bioremediated soil (11.6 %) and the C^{cs}_{TPH} even remained constant during the investigation period. These findings suggest that the differences of the degradation rates in the two soils do not result from the unequal initial contaminant concentrations but presumably are due to their different residence times in the soil. As presented in Chapter 1, the bioavailability of residual contaminants in soils decreases with increasing residence time due to *aging effects*. These are chemical reactions which incorporate the contaminants into natural organic matter or slow diffusion into very small pores and absorption into organic matter [Burgos et al., 1996; Karimi-Lotfabad et al., 1996]. As a consequence, a smaller amount of contaminants is available for microorganisms and extraction [Weissenfels et al., 1992; Alexander, 1995; Hatzinger and Alexander, 1995; White and Alexander, 1996].

From the field as well as the laboratory studies it can be concluded that the residual contaminants in the bioremediated soil can be further degraded, but only to a small extent.

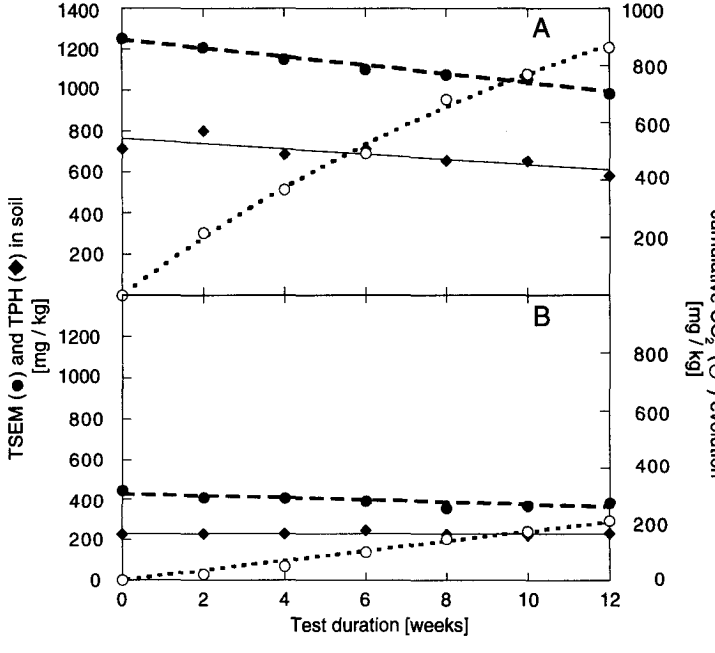

Figure 3-4 *Time course of CO_2 production and TSEM and TPH concentrations during the biodegradation study in: A: bioremediated soil which had been additionally spiked with residual contaminants; B: bioremediated soil.*

3.3.3 Volatility of residual contaminants

The concentration of the compounds in the soil air were too low to be analyzed by headspace with a quantitation limit of 2 $mg \cdot m^{-3}$ at 80°C (data not shown). Hydrocarbons with lower boiling temperatures (< 280°C) can easily be lost into the atmosphere due to volatilization during the excavation of the soil as well as the homogenization activities during the bioremediation process. Furthermore, these compounds are known to be eliminated faster during the biodegradation process than hydrocarbons with higher boiling temperatures [Oudot, 1984; Kennicutt, 1988]. Therefore, hydrocarbons with lower boiling temperatures are expected to be completely biodegraded during the remediation process of this soil [Oudot, 1984; Kennicutt, 1988].

3.3.4 Leaching behavior of residual contaminants

The leaching behavior of the residual contaminants was investigated by conducting two medium-scale (columns, filled with bioremediated soil and spiked quartz sand) and one large-scale experiment (lysimeter, filled with bioremediated soil). The experimental data is summarized in Table 3-1. To be able to compare the results obtained from the three leaching experiments, the resulting concentrations of residual contaminants in the leachates were normalized to the corresponding water/soil ratio ([w/w]). The watering rate in Column A as well as in the lysimeter, both filled with bioremediated soil, varied significantly in order to investigate the desorption kinetics of the residual contaminants therein. Since strong sorption onto the organic soil fraction and the increased residence time of the contaminants both could result in lower leaching rates, Column B was filled with freshly spiked quartz sand. The TSEM and TPH concentrations therein were slightly higher than the corresponding concentrations in Column A and the lysimeter (Table 3-1). Figure 3-5 shows that despite the very different experimental setups, the results from all the leaching experiments were very similar. While the initial concentrations of the residual contaminants in the leachates were elevated, they decreased rapidly with

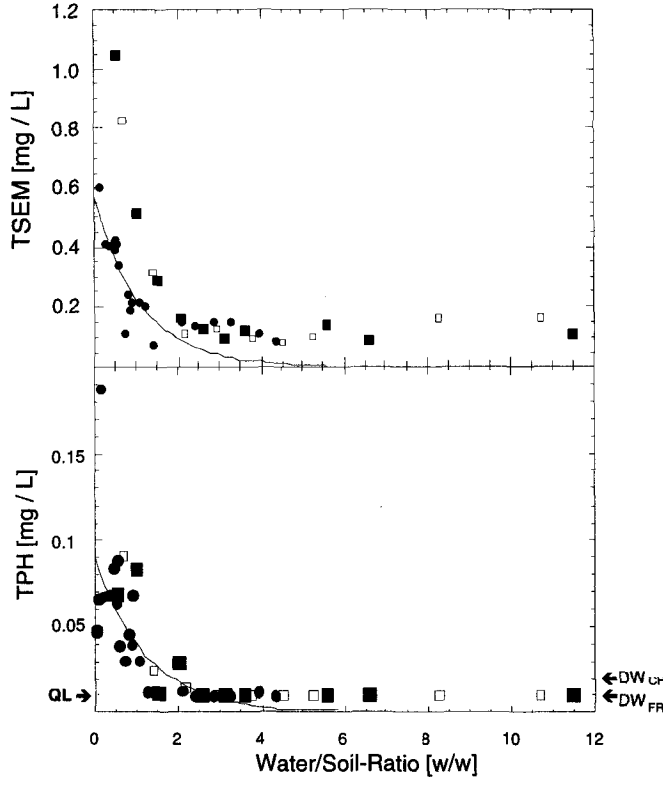

Figure 3-5 *TSEM and TPH concentrations in leachates as a function of the water/soil ratio from: ● Lysimeter, filled with bioremediated soil; □ : Column A, filled with bioremediated soil; ■ : Column B, filled with spiked quartz sand. The solid lines are exponential fittings of the leaching data from the lysimeter. The quantitation limit (QL) of the TPH in leachates was 0.01 mg·L^{-1}. Therefore, all concentrations below 0.01 mg·L^{-1} are set equal to the QL. DW_{CH}: Swiss drinking water standard: 0.02 mg·L^{-1}; DW_{FRG}: German drinking water standard: 0.01 mg·L^{-1}.*

an increasing water/soil ratio. It appears therefore, that the very low emissions of residual contaminants from bioremediated soil are probably due to the very low water solubility of the contaminants rather than due to their desorption kinetics or their sorption onto the organic matter in the soil. The C^{cs}_{TPH} decreased below the Swiss drinking water standard of 0.02 $mg \cdot L^{-1}$ [Eidg. Gesundheitsamt, 1988] at water/soil ratios of ≈ 2 and ≈ 3 for the lysimeter and the column studies, respectively. Both ratios correspond to an approximate precipitation of one year. At a water/soil ratio of 3, corresponding to a precipitation period of 1.5 years or less in all the leaching experiments, both the quantitation limit and the German drinking water standard of 0.01 $mg \cdot L^{-1}$ were achieved. Within the first 5 weeks of the investigation, 1.05 $mg \cdot kg^{-1}$ and 1.1 $mg \cdot kg^{-1}$ of TSEM were leached from Column A and Column B, respectively. These leaching rates correspond to a TSEM loss of 0.2 % in the first year for both columns. Similar results were also obtained from the lysimeter experiment. 0.92 $mg \cdot kg^{-1}$ or 0.12 % of the C^{s}_{TSEM} were determined to be lost due to leaching within the first year of precipitation. For the subsequent years, much lower leaching emissions are expected, since leaching rates decrease exponentially with increasing water/soil ratios (Figure 3-5). As expected from the physicochemical characterization of the residual contaminants in Chapter 2 [Angehrn et al., 1998], the water solubilities, and therefore the concentrations in the leachates, are very low. As presented in Table 3-1, the DOC values in column A and in the lysimeter are much higher than in column B. This indicates that the elevated DOC values are not related to the TSEM but could be due to organic components which are naturally occurring in these soils. Since the concentrations of the residual contaminants in the soil leachates were too low for their potential biodegradability to be directly determined, the CEC degradation test [CEC, 1995] was employed. As presented in Table 3-2, 20 % of the total residual contaminants were biodegraded in liquid medium after the 21 day test period. The results indicate that at least part of the TSEM in the leachates could be further biodegraded.

Table 3-2 *TSEM recovery after the 21 day CEC degradation test [CEC, 1995]. The standard deviation from triplicates is given in parentheses.*

Samples	TSEM recovery after 21 days [%]	corresponding control [1]
TSEM from bioremediated soil	76 (7.9)	95 (1.0)
Reference oil [2]	10 (1.6)	102 (2.1)

[1] *control was poisoned with $HgCl_2$ (0.03 mol*L^{-1})*
[2] *Di-iso-tridecyl adipate*

3.3.5 Mass-flow analysis

In order to evaluate the potential emissions of TSEM into the environment, a mass-flow analysis was performed for the first year after applying bioremediated soil as top soil. It is based on both experimental data presented in this study and model calculations obtained from the literature. The TSEM losses are illustrated in Figure 3-6.

Biodegradation and aging effects are expressed as a single term since these processes could not be investigated separately in the field study. The natural attenuation for a certain time period can therefore be written as:

$$M_{NA} = M_V + M_{PI} + M_L + M_{B/A} \qquad (3\text{-}1)$$

where M_{NA} is the total decrease of TSEM in the soil and M_V, M_{PI}, M_L and $M_{B/A}$ are the corresponding losses due to volatilization, plant uptake, leaching and biodegradation and/or aging effects, respectively. Illustrative calculations were

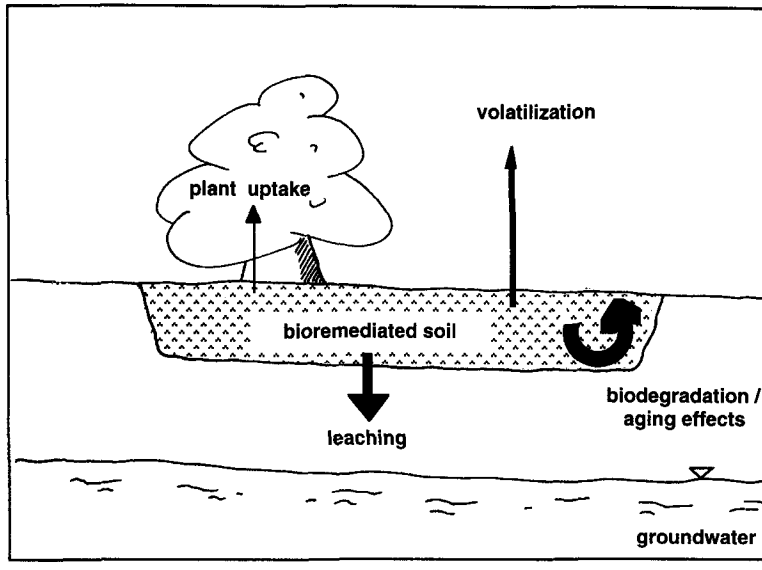

Figure 3-6 *Schematic illustration of the various transport and transformation processes included in the mass-flow analysis.*

made for a 0.5 m top soil layer for the first year after soil application, using n-octadecane as a reference compound. n-Octadecane is one of the most mobile constituents within the TSEM [Chapter 2; Angehrn et al., 1998] and therefore was used for these worst case calculations. The natural attenuation of TSEM (M_{NA}) was determined from the field experiment with an average TSEM reduction of 55 $mg \cdot kg^{-1}$ for the first year. As indicated earlier, no volatile components could be detected in the headspace analyses. The losses due to volatilization as a result of the molecular diffusion were estimated using a model presented by Little et al. [1992]. For these calculations, the following assumptions were made: (i) the water content in the soil was negligible (two phase model, with $\theta_v=\theta_t$), (ii) equilibrium

always exists between the solid and the gaseous phase, (iii) volatilization is limited by molecular diffusion, and (iv) no biodegradation occurs. The TSEM mass-flow (M_v) is based on the rate of vaporization (r_v) [Thibodeaux, 1979] as indicated below:

$$M_V = r_v \cdot SA \cdot \tau \tag{3-2}$$

where SA is the surface area, τ is the averaging time and r_v is:

$$r_v = \frac{D_{eff}}{x}(c_v - c_v^{sa}) \tag{3-3}$$

x is the layer thickness where the contaminants evaporated and where a concentration gradient exists in the soil. c_v is the average vapor concentration in the contaminated soil while c_v^{sa} is the vapor concentration at the soil/air interface and is set equal to zero. D_{eff} is the effective diffusion coefficient in the soil and calculated according to Little et al. [1992] as:

$$D_{eff} = \frac{D_{air}}{\theta_v \cdot R}\left(\frac{\theta_v^{3.33}}{\theta_t^2}\right) \quad \text{(3-4)}, \qquad \text{with the retardation factor} \quad R = 1 + \frac{\rho_s}{\theta_v} \cdot K_{sg}. \quad \text{(3-5)}$$

The soil vapor concentration c_v is:

$$c_v = \frac{c_{soil}}{K_{sg}} \quad \text{(3-6)}, \qquad \text{with the partition coefficient} \quad K_{sg} = \frac{K_{oc} \cdot f_{oc}}{H}. \quad \text{(3-7)}$$

Based on the values for the various parameters used in these equations (Table 3-3) as well as the physicochemical properties of n-octadecane, the annual TSEM loss due to volatilization was estimated to be $2.1 \cdot 10^{-4}$ mg·kg^{-1}. These calculations were based on the molecular diffusion alone and excluded the effects of wind and air pressure fluctuations. However, the low volatilization losses are due to the strong retardation of the highly hydrophobic, non-volatile compounds such as TSEM, the volatilization process is rather desorption-limited. Consequently, the TSEM decrease in bioremediated soils due to volatilization can be neglected.

Table 3-3 *Parameters employed in the illustrative calculations for the mass flow analysis using n-octadecane as reference compound.*

Notation	Parameter	Dimension	Value		Reference
Parameters used in the calculation for volatilization (equation 3-2) and plant uptake (equation 3-8)					
C_{soil}	soil concentration	$g_{TSEM} \cdot g_{soil}^{-1}$	$7.8 \cdot 10^{-4}$	m [1]	this study
C_v	soil vapor concentration	$g_{TSEM} \cdot L^{-1}$	$2.3 \cdot 10^{-10}$	c [2]	Little et al., 1992
C_v^{sa}	soil vapor conc. at soil/air interface	$g_{TSEM} \cdot L^{-1}$	0	a [3]	this study
x	uncontaminated soil layer	cm	0.001	a	this study
ρ_s	soil bulk density	$g \cdot cm^{-3}$	1.4	l [4]	ASTM, 1995
θ_w	vadose soil water content	$cm_w^3 \cdot cm_{soil}^{-3}$	0.12	l	ASTM, 1995
θ_t	total soil porosity	$cm^3 \cdot cm_{soil}^{-3}$	0.38	l	ASTM, 1995
r_v	rate of vaporization	$g_{TSEM} \cdot cm^{-2} \cdot s^{-1}$	$9.3 \cdot 10^{-18}$	c	Thibodeaux, 1997
D_{eff}	effective diffusion coefficient	$cm^2 \cdot s^{-1}$	$4.0 \cdot 10^{-9}$	c	Little et al., 1992
τ	averaging time (one year)	s	$3.2 \cdot 10^7$	a	this study
SA	surface area	cm^2	7.1	a	this study
f_{OC}	organic carbon fraction in soil	$g_{oc} \cdot g_{soil}^{-1}$	0.017	m	this study
SCF	stem concentration factor	-	$1.1 \cdot 10^{-7}$	c	Ryan et al., 1988
Y_{Pl}	plant yield	$g_{biomass} \cdot cm^{-2}$	0.153	l	Gisi, 1990
chemical-specific parameters for ***n-octadecane***					
K_{ow}	octanol/water partition coefficient	-	10^{10}	e [5]	Angehrn et al., 1998
K_{oc}	org. carbon/water part. coefficient	-	$10^{8.34}$	c	Schwarzenbach et al., 1993
K_H	Henry coefficient	-	1.1	l	Schwarzenbach et al., 1993
K_{sg}	soil solid/vapor partition coefficient	$L \cdot kg^{-1}$	$3.4 \cdot 10^6$	c	Little et al., 1992
D_{air}	diffusion coefficient in air	$cm^2 \cdot s^{-1}$	0.069	l	Schwarzenbach et al., 1993
R	retardation factor	-	$1.3 \cdot 10^7$	c	Little et al. 1992

1) *measured* 3) *assumption* 5) *estimated*
2) *calculated* 4) *literature data*

The decrease of the TSEM concentration in bioremediated soil due to plant uptake has not been addressed so far. It is difficult to quantify the plant uptake of TSEM analytically, since similar compounds occur naturally in plants [Dragun et al., 1990; Ripper et al., 1993; Stephanou and Stratigakis, 1993]. An estimation of the

magnitude of the TSEM loss due to plant uptake was made by calculating a Stem Concentration Factor (SCF) according to Ryan et al. [1988]. The SCF is the ratio of the concentration in the stem to the concentration in the soil, a parameter depending on the organic carbon/water partition coefficient (K_{oc}), the fraction of organic carbon in soil (f_{oc}), the soil water content (θ_w), and the soil bulk density (ρ_s). The corresponding parameters used for these calculations are also presented in Table 3-3. The TSEM loss due to plant uptake (M_{Pl}) was calculated as:

$$M_{Pl} = SCF \cdot C_{soil} \cdot Y_{Pl} \cdot \tau \qquad (3\text{-}8)$$

where Y_{Pl} is the average plant yield. M_{Pl} was calculated to be $1.9 \cdot 10^{-7}$ mg·kg^{-1} for the first year, based on the data for n-octadecane. Leaching of TSEM from bioremediated soil (M_L) was quantified by the results obtained from the lysimeter study, resulting in a total TSEM loss of 0.92 mg·kg^{-1} for the first year.

Leaching is the most important transport process for TSEM components in the soil. However, given the M_{NA} value of 55 mg·kg^{-1} for the first year, the mass balance in equation (3-1) indicates that $M_{B/A}$ should be approximately 54 mg·kg^{-1}. This suggests that biodegradation and/or aging effects are the main processes with respect to the natural attenuation of residual contaminant concentrations in bioremediated soil.

As presented earlier, however, the majority of the TSEM components (93 % after the first year) remained in the soil.

The TSEM decrease for 1 and 2.5 years as well as the roughly estimated TSEM emissions for 100 years are presented in Table 3-4. It is indicated by these extrapolation calculations that even after one hundred years, less than 2% of the initial TSEM components would be expected to be released into other environmental compartments. Since n-octadecane is one of the most mobile contaminant within the TSEM [Chapter 2; Angehrn et al., 1998], these above estimations can be considered to be very conservative.

Table 3-4 *Estimated TSEM decrease in bioremediated soil, based on the results obtained from this study, for different time scales. The corresponding cumulative emissions of the initial TSEM concentration from the soil are presented in [%].*

Processes	1 year			2.5 years			100 years		
	TSEM decrease		Source	TSEM decrease		Source	TSEM decrease		Source
	[mg*kg^{-1}]	[%]		[mg*kg^{-1}]	[%]		[mg*kg^{-1}]	[%]	
Natural attenuation (average)	55	7.1	field[1)]	139	17.8	field	n.d.[6)]	n.d.	
Transport processes									
volatilization	2.1*10^{-4}	<0.001	calc.[2)]	5*10^{-4}	<0.001	calc.[2)]	2.1*10^{-2}	0.003	calc.[2)]
leaching	0.92	0.12	Lys[3)]	1.35	0.17	extr.p.[7)]	12.01	1.54	extr.p.[7)]
plant uptake	1.9*10^{-7}	<0.001	calc.[4)]	4.7*10^{-7}	<0.001	calc.[4)]	1.9*10^{-5}	<0.001	calc.[4)]
Transformation processes									
biodegradation and/or aging effects	54	6.9	calc.[5)]	137	17.6	calc.[5)]	n.d.	n.d.	

[1)] determined from the field study

[2)] calculated by employing equation (3-2)

[3)] determined from the lysimeter study

[4)] calculated by employing equation (3-8)

[5)] calculated by employing equation (3-1)

[6)] not determined

[7)] extrapolated from data in Figure 3-5

4

bioassays using bioremediated soil and reference samples

Abstract

Even after successful bioremediation of mineral oil contaminated soils, some residual contaminants remain therein for a prolonged period of time. These compounds could adversely affect plants growing on these soils or, after they would be transported to surface waters due to water runoff or leaching, they could potentially harm water organisms. In order to determine the phytotoxicity potentially caused by these contaminants, plant growth studies were performed with 36 different plant species. No inhibitory effects could be determined for any of the plant species germinating and growing on bioremediated soil. Additional investigations with artificially contaminated soils revealed that no differences in plant growth and plant morphology appeared compared to the corresponding uncontaminated controls. The potential acute toxic effects of leached residual contaminants of bioremediated soils were investigated by conducting bioassays using standardized tests with *Vibrio fischeri* and *Daphnia magna*. From the results it can be concluded that the residual contaminants in bioremediated soils do not cause any acute toxic effects to these organisms. By comparing the results with those from various reference samples it is indicated that the residual contaminants in bioremediated soil have no acute toxic effects, neither on plants nor on selected water organisms.

4.1 Introduction

To assess the environmental risk potential of residual contaminants in environmental samples, bioassays have been shown to be useful since the effects of all contaminants, including those not considered in the chemical analyses, are integrated therein [Kreysa and Wiesner, 1995]. Analytically detected concentrations of contaminants in environmental samples do not necessarily provide a sure prediction of any harmful biological or ecological effect since the bioavailability can vary considerably depending on the chemical species and the environmental conditions [Kreysa and Wiesner, 1995].

In Chapter 3 it was demonstrated that the majority of the residual contaminants in bioremediated soil will remain therein for a prolonged period of time and therefore could adversely affect plant growth. Plants are very sensitive to soil contamination and must, therefore, be considered as part of an ecological risk assessment [Linder et al., 1990]. It is reported that plant growth was affected when mineral oil products had been freshly added to the soil [CONCAWE, 1996; Wang and Bartha, 1990; Salanitro et al., 1997; Bossert and Bartha, 1985]. In order to evaluate the phytotoxicity potentially caused by the residual contaminants remaining in bioremediated soil, plant growth studies were conducted with 36 different plant species. Since leaching was determined to be the major emission of the residual contaminants from the soil, the potential acute toxic effects of the leachates to water organisms were also assessed. The inhibition of light emission in *Vibrio fischeri* [DIN 38'412, 1991] and the immobilization of *Daphnia magna* [OECD, 1984] in soil eluates, two standardized tests which are widely accepted for assessing the acute toxicity of aquatic contaminants [Bobra et al., 1983; Ribo and Rodgers, 1990; Wang et al., 1990; Kreysa and Wiesner, 1995; Salanitro et al., 1997] have been applied. To be able to evaluate soil contamination as well as the associated acute toxic effects, background concentrations in the environment must be considered. Since no background concentrations existed for the investigated bioremediated soil, uncontaminated reference samples, either natural soils (forest, field, gravel) or

materials normally used to increase soil fertility (sewage sludge) or soil quality (compost, garden soil) were compared.

4.2 Material and methods

4.2.1 *Preparation of soil and reference samples*

Bioremediated soils were obtained from a remediation company after the treatment had been completed. Besides the bioremediated soil *Pfyn*, another bioremediated soil, named with *Felben*, was investigated in this chapter. Soil samples from a beech forest and a sunflower field were collected from the surface layer (5-15 cm). Gravel was obtained from a gravel-pit (grain size: 0.9 mm - 12 mm). One and nine months old compost samples were collected at a composting plant. Dried and granulated sewage sludge was obtained from a municipal wastewater treatment plant. The garden soil was purchased from a general supermarket. For chemical analyses, all samples were air dried and, except for the gravel, crushed and sieved (< 2 mm) before extraction. Spinach was oven dried (48h at 70°C) and subsequently pulverized in a centrifugal grinder. The reference soil employed for plant growth studies, a mixture of a variety of agricultural top soils, was obtained from a sugar beet washing plant. The concentrations in all the samples refer to the dry weight and all reference samples are assumed to represent uncontaminated material.

4.2.2 *Spiking the soils with TSEM*

For plant growth studies, soil samples as well as quartz sand were spiked with residual contaminants which previously had been soxhlet extracted from the bioremediated soil as described in Chapter 2 [Angehrn et al., 1998]. To apply the contaminants homogeneously onto the soil surface, it was necessary to dissolve these compounds in a volatile solvent. Approximately 10 mL of 1,1,2-trichlorotrifluoro-ethane was used for this procedure. To ensure equal distribution of the contaminants throughout the amended soils, the solution was sprayed onto the soil in three portions with thorough mixing of the soil after each application. The solvent was allowed to evaporate by mixing the soil periodically. Test results, using control soils

only treated with 1,1,2-trichlorotrifluoroethane, indicated no adverse effects from the solvent.

4.2.3 Analyses of TSEM and TPH in soil samples

The prepared soil samples (see above) were thoroughly mixed with anhydrous Na_2SO_4 (to absorb residual moisture) prior to soxhlet extraction with tetrachloromethane [Chapter 2; Angehrn et al., 1998]. Quantification of the TSEM and TPH concentrations was performed by infrared spectroscopy (IR) (Perkin Elmer, 1600 series, Breitenbach, Switzerland), using a Simard standard [Douglas et al., 1992]. The TPH concentrations were obtained after an alumina column cleanup to remove polar compounds [U.S.EPA, 1989] and subsequently quantified by IR. Analyses using high resolution gas chromatography (HRGC) were performed on a Fisions HRGC Mega II apparatus (CE Instruments, Rodano, Italy), equipped with a Flame Ionization Detector (FID) and a BGB-5 column (30m x 0.32 mm x 0.25 µm; BGB Analytik AG, Zürich, Switzerland). The column was kept at 80°C for 5 min, then heated to 300°C at a rate of $6°C \cdot min^{-1}$ and again kept at 300°C for 15 min. Cold on-column injection technique was employed and hydrogen was used as the carrier gas.

4.2.4 Analyses of TSEM and TPH in water samples

After acidifying the water samples using HCl conc. (pH <2) and after adding NaCl (5 g per 500 mL of water), the TSEM was extracted with 25 mL tetrachloromethane by shaking for one hour. The solvent phase was separated from the water phase in a separation funnel, dried with Na_2SO_4 and subsequently filtrated through glass wool. The TSEM concentration and subsequently the TPH concentration in the solvent were determined by IR as described above. The quantitation limit of TSEM and TPH in water samples was $0.01\ mg \cdot L^{-1}$.

4.2.5 Dissolved Organic Carbon (DOC) in water samples

5 mL of a water sample were filtrated (0.45 µm, cellulose membrane, Millipore, Volketswil, Switzerland), mixed with 100 µL 2M HCl and analyzed on a TOC-analyzer (Shimadzu; TOC-5050, Burkhard Instruments, Zurich, Switzerland). The quantitation limit was 1 mg $C \cdot L^{-1}$.

4.2.6 Eluate tests

Eluate tests were conducted according to DIN 38414 [1984]. This method initially was developed for the determination of water soluble compounds in mud and sediments with a ratio of solids to water of 1:10. In contrast to the guideline, a ratio of solids to water of 1:2 was used as proposed by Kreysa and Wiesner [1995] for investigations with soils. This ratio represents a more realistic simulation of the natural soil conditions and the eluates are less diluted. After 24 hours, the mixture was centrifuged (10'000·g at 20°C; 15 min) and filtrated using membrane filters (0.45 µm, cellulose membrane, Millipore, Volketswil, Switzerland). The contaminants were extracted from the clear supernatants as described above.

4.2.7 Plant growth experiments

To evaluate bioremediated soil as substrate for plant growth, seeds from 36 plant species (both monocotyledonous and dicotyledonous plants) were added to this soil and seed germination after 10 days as well as the morphology (necrosis, chlorosis, stunted growth) of the growing plants after 30 days was investigated in a greenhouse experiment. The investigated plants, a selection of cultivated crops and weeds, are listed in Table 4-1.

Table 4-1 *Codes and corresponding plant names for the 36 plant species investigated in the plant growth studies.*

Code		Plant names	Code		Plant names
ABTHE	[1]	*Abutilon theophrasi*	HEANN	[1]	*Helianthus annuus*
ALMYO		*Alopecurus myosuroides*	HOVUL	[1]	*Hordeum vulgare*
AMRET	[1]	*Amaranthus retroflexus*	MACHA		*Matricaria chamomilla*
APSPV		*Apera spica venti*	PAMIL		*Panicum miliaceum*
AVFAT	[1]	*Avena fatua*	POANN		*Poa annua*
BETVU	[1]	*Beta vulgaris alt.*	POOLE		*Portulaca oleracea*
BRAPL		*Brachiaria platophylla*	SEVUL		*Senecio vulgaris*
BRTEC	[1]	*Bromus tectorum*	SEFAB	[1]	*Setaria faberi*
CABUR		*Capsella bursa-pastoris*	SEVIR		*Setaria viridis*
CAOBT		*Cassia obtusifolia*	SISPI		*Sida spinosa*
CHALB		*Chenopodium album*	SINAL	[1]	*Sinapis alba*
CYROT		*Cyperus rotundus*	SONIG	[1]	*Solanum nigrum*
DATST		*Datura stramonium*	SOHAL		*Sorghum halepense*
DISAN		*Digitalis sanguimalis*	SOVUL	[1]	*Sorghum vulgare*
ECCRU	[1]	*Echinochloa crus-galli*	STMED		*Stellaria media*
GAAPA		*Galium aparine*	TRSAT	[1]	*Triticum sativum*
GLYMA	[1]	*Glycine max*	XAPEN		*Xanthium penssylnanicum*
GOSHI	[1]	*Gossypium hirsutum*	ZEMAY	[1]	*Zea mays*

[1] *Plants for which biomass production in contaminated and uncontaminated soil and quartz sand was compared.*

For control purposes, the same plants were grown on a reference soil. This test soil was a mixture of fertile agricultural top soils while the bioremediated soil was predominantly excavated material from the underground. Due to the very different origins of the two soils, seed germination and plant growth but no plant yield determinations were investigated in this experiment.

To investigate directly the plant yield in soils with and without residual contaminants from bioremediated soil, test soils were spiked with residual contaminants from the bioremediated soil as described above and the plant weight of eight cultivated crops as well as eight weeds were compared. The methodology used in these plant growth

studies was similar to that outlined in the OECD guideline [OECD, 1984]. Two sets of different soils were investigated, using (a) reference soil and (b) quartz sand (prewashed; grain size: 0.3 mm - 0.9 mm). Each test set consisted of (i) soil which was spiked with residual contaminants from bioremediated soil, (ii) soil which was treated only with solvent, and (iii) untreated soil. The reference soil or quartz sand was dispensed into non-porous plastic nursery pots (300 cm^3). To each nursery pot, 4 to 50 plant seeds were added, depending on the seed sizes. The pots were kept in a greenhouse and watered daily. To the pots with quartz sand, liquid fertilizer was added to the water to ensure sufficient nutrients for the plants. Each treatment was prepared in triplicates. After 30 days, the plants from one pot were harvested by removing all plants above the soil surface as a group and their fresh weight was determined gravimetrically immediately thereafter.

4.2.8 Bioassays using water organisms

Kreysa and Wiesner [1995] recommended test methods that are considered suitable to evaluate the toxic potential of treated soils before they could further be reused. Two of the suggested test procedures which are also regulated and standardized by DIN [DIN 38412, 1991] and OECD [1984] were employed to evaluate the potential adverse effects of the dissolved residual contaminants in aqueous soil extracts:

Luminescent bacteria test: These tests were performed by employing a standard procedure outlined in DIN 38412 [1991]. For this purpose, a LUMIStox test set (Dr. Lange, Dusseldorf, Germany) was used. The test is based on the bioluminescence of reconstituted freeze-dried *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum*) as a measure of biological activity. Differences in light emission by bacteria due to the addition of the potential toxicants in the soil eluates were determined in duplicates at 15°C test temperature. Thirty-minutes test results were used for five exposure concentrations. The inhibitory effects on these organisms are expressed as the G_L value. This is the lowest value of the dilution factor G, for which the test gives a reduction in light emission of less than 20 % [Kreysa and Wiesner,

1995]. The sample is considered toxic, when the G_L value is > 8 [Kreysa and Wiesner, 1995].

Tests with Daphnia magna: A standard procedure outlined by OECD [1984] was used to evaluate the potential toxic effects in soil eluates to the water flea (*Daphnia magna STRAUS*). The organisms (≤ 24 hours old) used in these toxicity tests were from laboratory stocks cultured at BMG Engineering AG (Schlieren, Switzerland). The water was prepared using deionized, O_2-saturated water to which a total Ca- and Mg-ion content of 2.5 mmol·L^{-1} and ratios of Ca- to Mg-ions and Na- to K-ions of 4:1 and 10:1, respectively were added. The test duration was 48 hours. Four different exposure concentrations for each soil eluate plus a control were investigated.

4.3 Results and discussion

4.3.1 Contaminants in various soil and reference samples

Compared to the TSEM concentration in bioremediated soil, the corresponding concentrations in the reference samples, except for gravel, are similar (forest and field soil) or much higher, as presented in Table 4-2. From the high TSEM concentrations in the uncontaminated samples it appears that the multitude of chemicals analyzed by these surrogate parameters are compounds other than petroleum hydrocarbons which occur naturally in soils, such as e.g. humic substances.

No correlation between the TSEM and TPH concentration in a sample exists. Although the TSEM concentration in most reference samples were above that in bioremediated soils, most of the corresponding TPH concentrations were lower, by a factor of 2 to 10. Furthermore, no predictions can be made from the TSEM or TPH concentration in the solid matter for the corresponding concentrations in the aqueous soil eluates. Therefore, neither the leaching behavior nor the amount of leached TPH contaminants can be predicted from the corresponding concentrations in soils. HRGC chromatograms from selected samples are presented in Figure 4-1. All samples show the typical UCM at higher HRGC retention times, caused by numerous unresolved constituents. Distinct signals, which have not been identified, are only present in the chromatograms from the reference samples but not in those from the bioremediated soils.

Table 4-2 *Surrogate parameters determined in this study from bioremediated soils and various reference samples as well as the corresponding soil eluates [DIN 38414, 1984]*

Investigated materials	Concentrations				DOC of eluates	*V. fischeri*	*D. magna*
	in soil [mg*kg^{-1}]		in soil eluate [mg*L^{-1}]		[mg*L^{-1}]	G_L [1]	Immobilization [2]
	TSEM	TPH	TSEM	TPH		[-]	[%]
Bioremediated soils							
"Pfyn" [3]	445	299	0.26	0.15	6.5	8	10.0
"Felben"	545	270	0.42	0.15	15.0	2	2.5
Reference samples							
beech forest top soil (0-15 cm)	463	21	0.23	0.01	15.9	2	5.0
gravel	37	20	0.07	< 0.01	6.2	4	0.0
sunflower field top soil (0-15 cm)	281	57	0.09	0.03	16.6	8	2.5
garden soil (supermarket)	29'869	432	0.68	0.06	107.7	4	0.0
compost (age: 1 month)	4'271	142	1.00	0.13	301.3	16 [4]	52.5 [4]
compost (age: 9 months)	4'121	160	0.89	0.10	292.4	16 [4]	17.5 [4]
spinach	28'152	1'613	1.36	< 0.01	5'292.0	n.d. [5]	100.0 [4]
dried sewage sludge	26'733	6'744	5.42	0.16	1'473.0	32 [4]	17.5 [4]

[1] *Dilution factor for which the test, according to DIN 38412 [1991], gives a reduction in light emission for Vibrio fischeri of less than 20 %.*

[2] *48h test according to OECD [1984], values ≤10 % are not significant*

[3] *soil used for experimental investigations throughout this study*

[4] *elevated values due to secondary effects (such as O_2 shortage, elevated ionic strength)*

[5] *not determined*

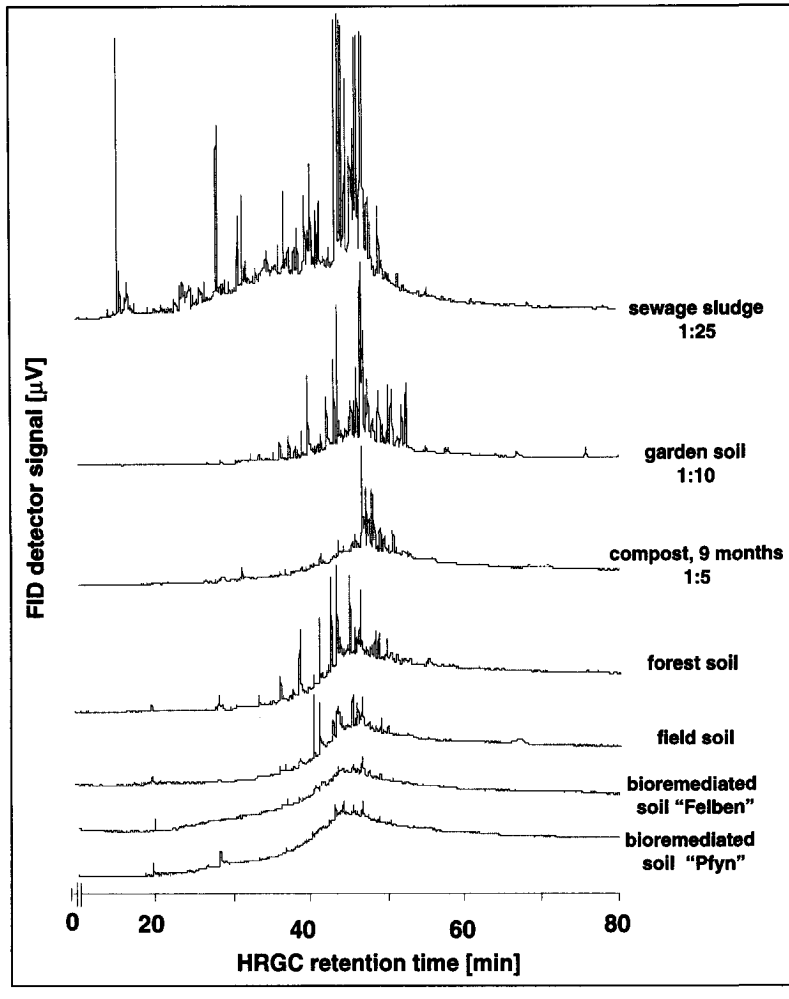

Figure 4-1 *HRGC chromatograms of soxhlet extracted TSEM from selected reference samples. The upper three chromatograms are presented for diluted probes, with the dilution ratio given below the sample names.*

4.3.2 Plant growth

To evaluate the potential risk caused by the residual contaminants remaining in bioremediated soil, seed germination and plant growth of 36 plant species presented in Table 4-1 were investigated on both, bioremediated soil as well as an uncontaminated reference soil. Seed germination and growth of all plant species were comparable in both soils after the first ten days of investigation. After the 30 day investigation period, none of the plant species grown on bioremediated soil showed any interference in plant morphology, with some weeds (*Matricaria chamomilla, Senecio vulgaris, Sinapis alba, Stellaria media*) developing flowers on both soils. A selection of plants are presented in Figure 4-2.

Figure 4-2 *Twelve of the 36 plant species growing on bioremediated soil which were investigated in greenhouse experiments in triplicates. The name of the plants are presented in Table 4-1.*

These results indicate that the residual contaminants in bioremediated soil had no toxic effects on the investigated plants.

In order to determine potential phytotoxic effects of the residual contaminants extracted from bioremediated soil, a reference soil was spiked with these contaminants. The concentrations of TSEM and TPH in the spiked soil was 1'270 $mg \cdot kg^{-1}$ and 330 $mg \cdot kg^{-1}$, respectively. The growth of 16 plant species (8 cultivated crops, 8 weeds) and the plant yield was compared with that in an uncontaminated control (840 mg $TSEM \cdot kg^{-1}$ and 70 mg $TPH \cdot kg^{-1}$). The weights of the harvested plants after 30 days were recorded and are presented in Figure 4-3.

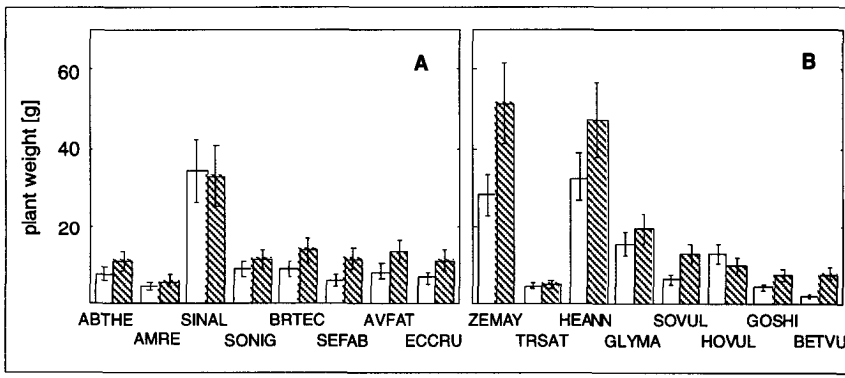

Figure 4-3 *Plant weight of (A) weeds and (B) cultivated crops grown for 30 days in a reference soil : ☐ uncontaminated control; ▨ soil spiked with residual contaminants from bioremediated soil. The codes for the different plant species are presented in Table 4-1. The standard deviation is expressed by error bars.*

The plant weights for most plant species, except for *Sinapis alba* and *Hordeum vulgare*, were higher in spiked quartz sand than in the uncontaminated control and no morphological alteration occurred during the test period. From these results it is indicated that the presence of the residual contaminants in the spiked soil did not cause any phytotoxic effects on the plants investigated in this study. Similar results have been reported in the literature. Wang and Bartha [1990] and Salanitro et al. [1997] described that bioremediated soil, after 5 to 11 months of soil remediation, again supported plant cover after an initial growth inhibition.

Due to the strong sorption of the organic contaminants onto the soil organic matter [Schwarzenbach, 1993], the availability of the residual contaminants for plant roots could have been strongly reduced during the 30 day experiment. In order to minimize this potential effect, identical experiments were performed with spiked quartz sand (470 mg TSEM·kg^{-1} and 290 mg TPH·kg^{-1}) as well as the corresponding uncontaminated control (20 mg TSEM·kg^{-1} and 13 mg TPH·kg^{-1}), using the same 16 plant species. Compared to the experiment with the reference soil, the growth of most plants was reduced in quartz sand, except for two plant species (*Echinochloa crus-galli, Zea mays*). This can be explained by the increased water content in the sand which would rather simulate a water saturated location and therefore present unfavorable growth conditions for many of the investigated plant species [Landolt, 1977]. However, all the seeds germinated and the plants did not show any morphological alterations. As presented in Figure 4-4, the weight for most of the harvested plants after 30 days were higher in spiked quartz sand than in the uncontaminated control. Therefore it is indicated that no phytotoxic effects were caused by the residual contaminants, a result which is in agreement with data presented in the literature. The growth of ryegrass (*Lolium perenne*) was inhibited when seeds were sewn directly in soils which were spiked with n-alkanes and PAHs [Günther et al., 1996]. Furthermore, germination of seeds was adversely affected by the presence of fresh hydrocarbons in the soil and plant growth was significantly reduced (< 80 %) [Chaineau et al., 1997]. However, when petroleum hydrocarbons

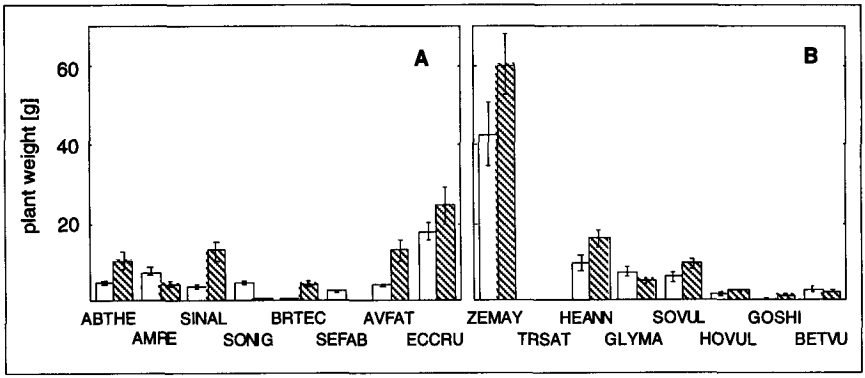

Figure 4-4 *Plant weight of (A) weeds and (B) cultivated crops grown for 30 days in quartz sand: ☐ uncontaminated control; ▨ quartz sand, spiked with residual contaminants from bioremediated soil. The codes for the different plant species are presented in Table 4-1. The standard deviation is expressed by error bars.*

are degraded in the rhizosphere, the toxicity of the residual oil contaminants is reduced and plants developed without interference, even if nondegradable hydrocarbons remain in the soil [Bossert and Bartha, 1985; Wang and Bartha, 1990; Hund and Traunsperger, 1994; Salanitro et al., 1997; Chaineau et al., 1997]. Because the lighter and more mobile constituents in fresh crude oils and mineral oil products tend to weather faster, the constituents remaining in weathered crude oils and mineral oil products are typically less mobile and, thus, can pose a lower risk than fresh products [Gustafson, 1998b].

4.3.3 Bioassays using water organisms

Luminescent bacteria test : For evaluation of soils, this test should be viewed as a screening test which may indicate a toxic effect on the bacteria as the measured light emission is a physiological indicator which is not necessarily correlated to the metabolic process [Kreysa and Wiesner, 1995]. However, the relative toxicity of different soil eluates to these organisms can be evaluated. The results obtained for eluates from bioremediated soils as well as from reference samples in the 30 min bioluminescence test are presented in Table 4-2. Compared to the soil eluates of reference samples from various uncontaminated locations, no toxic effects to these water organisms could be determined for bioremediated soils. This result is supported by the study of Salanitro et al. [1997], who recorded that crude oil contaminated soils lose their inhibiting activity on *Vibrio fischeri* after three months of bioremediation. Similar results were presented by Wang and Bartha [1990] and Wang et al. [1990] who reported full soil recoveries within 20 weeks after fuel spills with jet fuel, heating oil as well as diesel fuel.
The reduction in light emission in some reference samples (compost samples and sewage sludge) should not be interpreted as a toxic effect associated with the residual contaminants, since samples which contain easily degradable organic substances may cause a reduction in bioluminescence [Kreysa and Wiesner, 1995].

Tests with Daphnia magna: The 48 hours test results with soil eluates from bioremediated soils as well as reference samples are also presented in Table 4-2. As is evident, no acute toxic effects to these organisms could be observed for eluates of bioremediated soils. Furthermore, the experimental results for bioremediated soils are comparable to those obtained for eluates of uncontaminated reference samples. These results are consistent with that from Bobra et al. [1983] who reported that with increasing weathering, the composition of weathered crude oils change and, as a consequence, the remaining compounds are less soluble in water compared to those in the fresh product, leading to a reduced toxic potential for *D. magna*. The acute toxicity of fresh and weathered Norman Wells crude oil to *D. magna* (48h LC_{50} at

20°C) was experimentally determined to be 2.0 (1.1-3.6) mg/L and 3.4 (1.4-5.2) mg/L, respectively [Bobra et al., 1989]. The acute toxicity of gasoline and diesel fuel to *D. magna* (48h EC_{50}) of the water accommodated fraction (3 days stirring) was 4.9-6.3 mg/L [CONCAWE, 1992] and 20-210 mg/L [CONCAWE, 1996], respectively. By comparing the data for fresh mineral oil products with the leachate concentrations (Figure 3-5) or soil eluate concentrations (Table 4-2) for weathered mineral oil products determined in bioremediated soils, no acute toxicity must be expected.

In the soil eluates from reference samples with high DOC values (compost samples, spinach and sewage sludge), a significant increase in immobilization of *D. magna* (up to 100 %) was found (Table 4-2). This can be explained by a rapid oxygen decrease in the test samples within the test period and by the elevated ionic strength, two control parameters which were monitored during these tests. The toxic effects would therefore rather depend on the test system used than on the presence of toxic compounds within these samples. An exception could be the sewage sludge sample, where a variety of toxic compounds could be present at high concentrations [Torstenson and Pettersson, 1996].

Based on the results obtained by these bioassays and on literature data it can be concluded that elevated TSEM and TPH concentrations in soils or soil eluates do not necessarily cause acute toxic effects to water organisms. Compared to the data obtained with uncontaminated reference samples, no acute toxic effects on water organisms could be determined for residual contaminants in the bioremediated soils. Furthermore, it appears from the data in Table 4-2 and the chromatograms in Figure 4-1 that no environmental hazards must be expected from the residual contaminants since the results for the uncontaminated reference samples are comparable or even higher than for the bioremediated soils. As reported by Riis et al. [1996], weathered mineral oil constituents had no acute toxic effects, a result which supports the findings from this study.

In contrast to the plant growth study, the tests with aquatic organisms represent only a short period of lifetime. Therefore, the determination of the acute toxicity using bioassays can only give limited information on the overall potential adverse effects of

contaminants in water samples to the test organisms since *chronic* effects, that is symptoms which are usually observed after an extended period of time, can not be detected. Furthermore, extrapolations to other organisms are uncertain since the mechanism of toxic action are not known [La Grega, 1994] and can also vary between different species [Moriarty, 1983]. Using only a limited variety of test species therefore can be insufficient to evaluate the overall toxicity of contaminated soil samples and the corresponding soil eluates. Nevertheless, if the results from toxicity tests using contaminated soil samples can be compared to the corresponding results obtained from uncontaminated reference samples, the relative toxicity of this sample can be determined.

5

toxicity assessment and risk characterization

As mentioned in Chapter 1, *toxicity assessment* as well as *risk characterization* are the last two stages in the course of the *risk assessment* process for the residual contaminants in bioremediated soil. In addition to the experimental approach in Chapter 4 which revealed that no acute toxic effects to the test organisms are caused by the residual contaminants from bioremediated soil, *Preliminary Remediation Goals* (PRGs) were calculated, based on the most current EPA toxicological and risk assessment information [U.S.EPA, 1996a]. PRGs combine current U.S. EPA toxicity values with standard exposure factors to estimate contaminant concentrations in environmental media (soil, air, and water) that are protective of humans, including sensitive groups, over a lifetime [U.S. EPA, 1996a]. The toxicity potential for humans

was calculated by using chronic RfD values introduced by the U.S. EPA [1996a]. The inhalation RfD (RfD_i) is used for those situations where there is continuous exposure to an ambient air contaminant and the oral RfD (RfD_o) is used where a contaminant is incorporated by ingestion [Paustenbach, 1997]. The calculated concentrations subsequently were compared to contaminant concentrations determined in the test systems in this study (Chapters 3 and 4) in order to assess the potential risk.

As demonstrated in Chapter 2, the majority of the residual contaminants can be represented by n-alkanes. n-Hexane is the most toxic n-alkane since it is the only n-alkane which can be biotransformed into a γ-diketone (2,5-hexanedione), a characteristic chemical structure which is associated with specific toxic effects to mammals [Stacey, 1995]. There is a swelling of the axons, demyelination and degeneration of the nerve fibers of the peripheral nervous system which generally can recover but only over a long time [Stacey, 1995]. Therefore, n-hexane was employed as *worst case* reference compound to calculate PRGs, although it can be assumed that only n-alkanes with $n > 16$ are commonly present in bioremediated soil [see Chapter 2; Angehrn et al., 1998].

Since a receptor can potentially be affected by more than one exposure pathway, the *hazard index*, calculated by adding the *hazard quotients* of each exposure pathway, must be further determined. The hazard quotients themselves are determined by the ratio of the estimated intake levels and the corresponding PRGs [La Grega, 1994]. This approach assumes that multiple, subthreshold exposures may result in an adverse effect and that the effects are additive [La Grega, 1994]. A hazard index of one or less is generally considered safe and a ratio greater than one suggests further evaluation [U.S. EPA 1996a].

5.1 Toxicity assessment

5.1.1 Toxicity estimations: QSARs

The assessment of aquatic toxicity of environmental contaminants is severely hampered by lack of experimental toxicity data. A very promising concept for estimating narcosis type toxicity exists, called QSAR, short for Quantitative Structure-Activity Relationships [Hermens, 1989]. Based on the work by Hermens [1989], four classes of chemicals were defined; (i) inert chemicals, (ii) less inert chemicals, (iii) reactive chemicals, and (iv) specifically acting chemicals [Verhaar et al., 1992]. The residual contaminants in bioremediated soils are assumed to be representatives of the inert chemicals which do not interact with specific receptors in an organisms. The mode of action of such compounds in acute aquatic toxicity is called *narcosis* [Verhaar et al., 1992]. Narcosis type toxicity is considered to be brought about by an absolutely nonspecific mode of action, in that the potency of a chemical to induce narcosis is entirely dependent on its hydrophobicity [Verhaar et al., 1992]. A chemical will, within certain boundaries, always be as toxic as its hydrophobicity (its log K_{ow}) indicates. However, since the log K_{ow} of the residual contaminants in the bioremediated soil range above 6, these boundaries ($0 < \log K_{ow} > 6$, [Verhaar et al., 1992]) are exceeded. Compounds that have a log K_{ow} higher than 6 do not normally exhibit acute toxicity, since they are generally taken up from water too slowly to show acute toxicity and some of these compounds are simply too bulky to be taken up through membranes. Of course this rule does not mean that compounds with log K_{ow} values outside this range are to be considered nontoxic, but only that it is not recommended to use narcosis type QSAR equations for modeling their toxicity [Verhaar et al., 1992].

Consequently, no QSARs were calculated but worst case calculations using toxicity data for a specifically acting chemical, i.e. n-hexane, were conducted as indicated below.

5.1.2 Calculations of the toxicity potential for humans

Ingestion of contaminants in drinking water: Illustrative calculations were performed in order to determine the environmental concentrations which would be of no harm to humans. The ingestion exposure to noncarcinogenic contaminants in drinking water, based on the oral RfD [U.S. EPA, 1996a], was calculated with the lowest possible dilution attenuation factor (DAF) of one [U.S. EPA, 1996b] as follows:

$$c\left[mg * L^{-1}\right] = \frac{THQ * BW_a * AT_a}{EF_r * ED_r * \left[\frac{IRW_a}{RfD_o} + \frac{VF_w * IRA_a}{RfD_i}\right]} \qquad (5\text{-}1)$$

The parameters used for this calculation are presented in Table 5-1, leading to an acceptable concentration of n-hexane in soil eluates of 0.35 mg·L^{-1} [U.S. EPA, 1996a]. Considering the fact that no volatile compounds remained in the bioremediated soil [Chapter 2; Angehrn et al., 1998], the volatilization term (VF_w·IRA_a/RfD_i) in equation (5-1) can be neglected [U.S. EPA, 1996a]. Consequently, concentrations of 2.2 mg·L^{-1} would be accepted for non-volatile compounds having the same toxicity as n-hexane. Moreover, 44 mg·L^{-1} in the soil eluates would be accepted if a default DAF of 20, which would account for natural processes that reduce contaminant concentrations in the subsurface [U.S. EPA, 1996a], is additionally used. The long-term health advisories for n-hexane in drinking water for children and adults are 4 and 10 mg·L^{-1}, respectively [U.S. EPA, 1996c]. However, the TPH concentrations in the leachates (Figure 3-4) and soil eluates from bioremediated soils, presented in Table 4-2, are 0.15 mg·L^{-1} and therefore well below these calculated concentrations for n-hexane.

No significant concentrations of specific chemicals of concern, such as U.S. EPA-PAHs and BTEX, were present within the bioremediated soil investigated in this study [Chapter 2; Angehrn et al., 1998] and the residual contaminants therefore could

Table 5-1 *Parameters used for illustrative calculations, obtained from [U.S. EPA, 1996a] and [U.S. EPA, 1996b].*

Parameters	Definition	Dimension	Default value
AT_a	averaging time (noncarcinogens) - adult	days	10'950
AT_c	averaging time (noncarcinogens) - child	days	2'190
BW_a	body weight - adult	kg	70
BW_c	body weight - child	kg	15
ED_c	exposure duration - child	yr	6
ED_r	exposure duration - residential	yr	30
EF_r	exposure frequency - residential	$day \cdot yr^{-1}$	350
IR_c	soil ingestion rate - child	$mg \cdot day^{-1}$	200
IRA_a	inhalation rate - adult	$m^3 \cdot day^{-1}$	20
IRW_a	drinking water ingestion - adult	$L \cdot day^{-1}$	2
THQ	target hazard quotient	-	1
VF_w	volatilization factor for water	$L \cdot m^{-3}$	0.5
Chemical-specific parameters: for ***n-hexane***			
RfD_i	reference dose - inhaled	$mg \cdot kg^{-1} \cdot day^{-1}$	$5.7 \cdot 10^{-2}$
RfD_o	reference dose - oral	$mg \cdot kg^{-1} \cdot day^{-1}$	$6.0 \cdot 10^{-2}$

be represented by n-hexane. If other contaminants with known toxic effects would remain in the soil, however, the corresponding data in equation 5-1 would be needed to evaluate the potential hazards caused by them.

Inhalation of contaminants in air : The inhalation exposure to noncarcinogenic contaminants in air was determined by using calculations suggested by U.S. EPA [1996a], again employing n-hexane as worst case reference compound:

$$c\left[\mu g * m^{-3}\right] = \frac{THQ * RfD_i * BW_a * AT_a * 1000 \mu g * mg^{-1}}{EF_r * ED_r * IRA_a} \qquad (5\text{-}2)$$

By using the default parameters from Table 5-1 in equation (5-2), concentrations of TPH in the air of 210 $\mu g \cdot m^{-3}$ would be acceptable. As presented in Chapter 3, direct headspace analyses revealed no volatile constituents within the residual contaminants in bioremediated soil and the corresponding calculations indicated that volatilization is negligible.

Ingestion of contaminants in soil : As presented earlier in Chapter 3, the majority of the residual contaminants remain in bioremediated soil for a long time period. In order to evaluate the general reuse of bioremediated soil, direct ingestion, one exposure pathway which has not been addressed so far, must be additionally determined. No experimental investigations were conducted on this topic, but conservative calculations were performed instead, based on the oral RfD for n-hexane [U.S. EPA, 1996b]:

$$c\left[mg * kg^{-1}\right] = \frac{THQ * BW_c * AT_c}{\frac{1}{RfD_o} * 10^{-6} kg * mg^{-1} * EF_r * ED_c * IR_c} \qquad (5\text{-}3)$$

The calculations for soil ingestion are based on the increased exposure during childhood [Calabrese, 1989], although it is believed that a six-year exposure for a child combined with a chronic RfD would be unnecessarily conservative [U.S. EPA, 1996b].

By employing default values from Table 5-1 in equation (5-3), the calculated acceptable concentrations in soils for children, using n-hexane as reference compound, would be 4'500 $mg \cdot kg^{-1}$. The TPH concentrations remaining in the bioremediated soil investigated in this study, however, is an order of magnitude lower than the calculated acceptable concentrations in soils.

If ingestion of residual mineral oil contaminants due to soil intake is investigated for bioremediated soil, one has to consider other potential intakes of comparable components as well. It is reported in the literature that plant oils contain alkanes with carbon numbers between C_{11} and C_{35} [Belitz and Grosch, 1992] at concentrations

between 0.01 and 0.5 % (v/v) [Schormüller, 1969]. Iso-alkanes, such as pristane and phytane, are naturally found in liver oil of marine animals (dogfish, codfish, herring, shark) as well [Belitz and Grosch, 1992]. According to Grob et al. [1992], petroleum hydrocarbons were found in salad-oils at concentrations up to 1'000 $mg \cdot kg^{-1}$. Furthermore, various packing materials for food, such as jute sacks, sisal sacks, plastic foils, and printed cardboard are containing petroleum hydrocarbons [Grob et al., 1993]. From cardboard, which is printed with ink containing 20-30 % petroleum hydrocarbons, the contaminants are transferred into the food [Droz and Grob, 1997]. Some food products with the corresponding concentrations of petroleum hydrocarbons are listed in Table 5-2.

Table 5-2 *Concentration of petroleum hydrocarbons in food samples*

Food samples	Concentration [$mg \cdot kg^{-1}$]			References
Packed in jute or sisal sacks				
hazelnuts	10	-	500	Grob et al., 1991
coffee		150		Grob et al., 1991
rice		100		Grob et al., 1992
almonds	10	-	200	Grob et al., 1992
milk chocolate (from cacao beans)	50	-	270	Grob et al., 1992
Packed in cardboard				
powdered milk, in paper bag	30	-	60	Droz, Grob, 1997
cereals	< 5	-	> 100	Droz, Grob, 1997
rice		60		Grob et al., 1992
honey, in waxed cardboard cup		300		Grob et al., 1992
Sweets				
various candies	1'000	-	3'500	Grob et al., 1992
chewing gums	2'000	-	45'000	Grob et al., 1992
Regulations				
U.S. food and drug administration, for specified lubricating oils for food (H_1 oil)		10		Grob et al., 1992
Swiss food control regulations		10		FIV, 1986

In various candies, petroleum hydrocarbons, mainly consisting of iso-alkanes with carbon numbers between C_{20} and C_{26}, are found at concentrations between 1'000 and 1'500 $mg \cdot kg^{-1}$ and 3'500 $mg \cdot kg^{-1}$ of petroleum hydrocarbons were found in a lollipop [Grob et al., 1992]. In order to compare these compounds with those present in soils, a HRGC chromatogram, adopted from Grob et al. [1992], is presented in Figure 5-1.

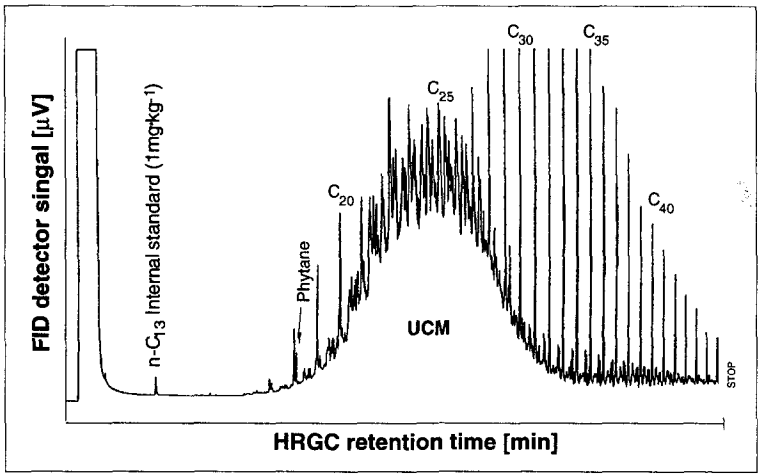

Figure 5-1 *HRGC chromatogram of petroleum hydrocarbons in a lollipop. The UCM and the resolved alkanes are produced by two different mineral oil products which were employed for production. Data adopted from Grob et al. [1992].*

If the UCM is compared to that from bioremediated soil in Figure 2-1 it is indicated that the mineral oil constituents forming the UCMs in both samples are within the same boiling range and it appears that very similar components are present in both samples. However, the concentration in the lollipop is much higher than the concentration in the soil. The highest concentrations of petroleum hydrocarbons, with

alkanes in the range between C_{20} and C_{40}, were found in chewing gums with 2'000-45'000 $mg \cdot kg^{-1}$ [Grob et al., 1992]. Although the hydrocarbons are not extracted by chewing, they can be incorporated when the gums are swallowed which often happens to children.

5.2 Risk characterization

The results from the exposure assessment in Chapter 3 as well as the toxicity assessment in this Chapter are summarized in Table 5-3.

The initial TPH concentration in undiluted leachates from the column and lysimeter tests described in Chapter 3 as well as the corresponding concentrations in the undiluted soil eluates (Table 4-2) are below the calculated PRG for n-hexane in drinking water. A hazard quotient of 0.43 can be calculated for a drinking water exposure, assuming that the leachate from the bioremediated soil would be incorporated as drinking water without further dilution. The concentration in the undiluted leachates, however, decrease below drinking water standards with increasing water/soil ratio (Figure 3-4), leading to a further reduction of the hazard quotient. Furthermore, the concentration of residual contaminants in the leachates would be further reduced due to the natural processes in the underground, such as dilution, degradation, dispersion, and adsorption [U.S.EPA, 1996a]. Since no acute toxic effects could be determined in plant growth studies for a variety of different plants (Chapter 4) and the plant uptake was calculated in Chapter 3 to be negligible, no adverse effects must be expected for plants. Furthermore, plant material analyses reveal high TPH concentrations as it is presented in Table 4-2 for spinach as well as in the literature for a variety of plants [Hollerbach, 1992; Ripper et al., 1993; Hellmann, 1995]. Therefore, the hazard quotient could be set very low. The concentration of residual mineral oil contaminants in ambient air was estimated in Chapter 3 to be negligible. Due to strong sorption processes onto the soil of the hydrophobic, non-volatile compounds, their volatility is very low. Consequently, the

Table 5-3 *Overview of compiled data presented in the exposure assessment (Chapter 3) as well as the toxicity assessment*

Environmental medium	Exposure assessment		toxicity assessment	Hazard quotient
Investigations	exp. [1]	calc. [2]	calc. [3]	[conc. / PRG]
Water				
TPH in leachates [4]	0.10 $mg \cdot kg^{-1}$		0.35 $mg \cdot kg^{-1}$	
TPH in soil eluates [5]	0.15 $mg \cdot kg^{-1}$			0.43
Air				
headspace meas.	< QL [6]			< 0.01
volatilization		negl. [7]	210 $\mu g \cdot m^{-3}$	
Soil				
soil ingestion [child]			4'500 $mg \cdot kg^{-1}$	
(TPH conc. in soil)	270 $mg \cdot kg^{-1}$			0.07
	Hazard index, the sum of all the hazard quotients :			**0.50**

1) *experimentally determined in this study*
2) *calculated according to ASTM [1995]; Table 3-5*
3) *calculated PRGs for n-hexane in residential soils according to U.S. EPA [1996a]*
4) *initial conc. in column and lysimeter study; Figure 3-4*
5) *according to DIN 38414 [1984]; Table 4-2*
6) *below quantitation limit (2 $mg \cdot m^{-3}$ at 80°C); Chapter 3*

hazard quotient was set very low. Based on the calculation of soil ingestion as well as the information about petroleum hydrocarbons in reference samples and food products, it can be concluded that direct ingestion of residual contaminants in bioremediated soils is not causing any health concerns. However, assuming that the bioremediated soil would be reused as top soil in a residential area, a resulting hazard quotient of 0.07 would be determined for soil ingestion. By adding the various hazard quotients of the potential exposure pathways for mineral oil contaminants in bioremediated soil, a hazard index of 0.50 is resulting. Even under the assumption that the residual contaminants in the bioremediated soil would possess a toxic

potential equal to that of n-hexane, the hazard index is below one. U.S.EPA [1996a] suggests that in general, these scenarios can be considered safe. Therefore it is indicated that a human would not be adversely affected by the residual contaminants from bioremediated soil, even if one would be exposed to various exposure pathways which actually is an *unrealistic* worst case scenario.

The toxicity of the residual contaminants was characterized by a single compound (n-hexane). This worst case approach probably significantly overestimates the toxicity and mobility of the residual contaminants [Gustafson, 1998b] because: i) n-hexane is the most toxic n-alkane; ii) n-hexane is a very mobile constituent in mineral oil products; and iii) n-hexane is not even present in the complex mixture of residual contaminants investigated in this study.

In Chapter 2, other compounds than aliphatics were determined by flash-chromatography and a significant fraction thereof was named with PAH-like compounds. However, less than 3 % of this fraction and subsequently less than 0.4 % of the TSEM were identified as EPA-PAHs [Chapter 2; Angehrn et al., 1998] which typically are considered as chemical of concerns (Table 1-4). The majority of the compounds within this fraction, however, is not known in detail and therefore no specific information on these compounds could be obtained. If the total amount of U.S. EPA-PAHs found in the bioremediated soil would be represented by the most toxic representative observed (i.e., benzo-k-fluoranthene), the acceptable soil concentration of 6.1 $mg \cdot kg^{-1}$ [U.S. EPA, 1996a] is an order of magnitude higher than the concentrations determined in the soil, and no adverse effects would therefore be expected from these components.

The findings of this study are supported by data reported in the literature. Soil cleanup guidelines for diesel fuels, based on an extended literature review on toxicity data as well as on calculations for ingestion, dermal, and inhalation routes for residents, were defined to be between 1'160 $mg \cdot kg^{-1}$ and 11'200 $mg \cdot kg^{-1}$, depending on the assumptions used [Millner et al., 1992]. Furthermore, the state regulatory agency of Michigan has adopted 10'000 $mg \cdot kg^{-1}$ as a generic risk based screening level for TPH at all crude oil contaminated sites, since the concentrations of the carcinogenic indicator PAHs are below levels of concern [Gustafson, 1998 b]. From

all of the above it can be suggested that the bioremediated soil investigated in this study could be widely reused without any restrictions.

Nevertheless, the results obtained from this study are not transferable without a proper re-evaluation on a case by case basis. Various parameters could deviate from those determined in this study, either due to different soil treatment conditions, e.g., remediation duration, or due to different contaminants present in soil. If specific components, which potentially could cause environmental concerns, can be identified within the complex mixture, additional investigations regarding these chemicals of concern must be addressed. However, the procedure of assessing the potential risks associated with residual contaminants in soils presented in this study could be adopted.

6

risk management

As mentioned in Chapter 1, the evaluation of cleanup levels for residual contaminants in soils are a major issue for regulatory agencies. For well defined individual chemicals, the physicochemical as well as the toxicological characterization and subsequently the determination of cleanup standards, such as the PRGs defined by the U.S. EPA [1996a], can be achieved. However, in the case of mineral oil products and the weathering products thereof, the determination of cleanup levels are causing major difficulties due to the lack of compositional as well as toxicological information. Nevertheless, decisions have to be made, based on presently limited information. Following the precautionary principles, the acceptable concentrations of residual mineral oil contaminants in soils, usually expressed by the TPH, are therefore presently set very low, and are used as conservative guidelines or standards by many regulatory agencies (Table 1-3). This approach, in the following called *TPH-approach,* is fast and seems to be protective for the environment in a comprehensive way since it allows to recognize easily the

potential problems by simply comparing the contaminant concentrations in the soils with the corresponding guidelines or standards. However, inherent drawbacks are connected to this TPH-approach which should briefly be addressed:

Determination of TPH : As discussed in Chapter 2, various extraction procedures and quantification methods exist for the determination of TPH in soils. As a consequence of the differing results which can be obtained by using these different procedures, the TPH concentrations determined in environmental samples and the corresponding guidelines or standards cannot be simply compared as long as the determinations are not based on equal analytical procedures. Therefore, as a minimal prerequisite, identical methods should be employed for all samples, not only for quantification but also for soil sampling, sample preparation and sample storage.

Natural TPH concentrations : From Table 4-2 as well as from literature data, [Hollerbach, 1992; Ripper et al. 1993; Hellmann, 1995], it can be concluded that uncontaminated reference samples may exhibit elevated TPH concentrations compared to guidelines or standards for TPH contamination in soil presented in Table 1-3. Consequently, background levels, caused by naturally occurring hydrocarbons (e.g. from plant material) and atmospheric depositions, would be designated by the TPH-approach as being of environmental concern although no mineral oil products have been spilled on these soils before.

Fresh vs. weathered mineral oil contaminants : By using this approach, no attention is paid to the processes affecting the composition of the residual contaminants in the soil (see Chapter 1). Soils freshly contaminated with mineral oil products can cause major environmental concerns due to the water solubility or the volatility of some of the constituents, i.e. monoaromatics (BTEX) or short-chain alkanes (e.g. n-hexane). As presented in this study, however, the chemical composition of mineral oil contaminants in soils change with increasing residence time. Consequently, the remaining complex mixtures, in contrast to the initial contaminants, possess different physicochemical as well as toxicological properties.

Disposal in landfills : According to the Swiss regulations based only on the TPH-approach, bioremediated soil with TPH concentrations above the corresponding guidelines have to be disposed of in landfills. As a consequence, the limited space in landfills would be used up by soil which actually could be reused for a variety of alternative applications.

Evaluation of soil remediation technique : From an economic point of view, the bioremediation of contaminated soils is attractive for the operator of this soil treatment technology only if the soil can be reused after the remediation process has been completed. If bioremediated soil needs to be disposed of in landfills, however, alternative remediation techniques most probably would be preferred to treat these soils. As a consequence, the soil quality would be highly reduced since the soil as a living system would be destroyed. Furthermore, new environmental pollutants could be produced due to increased transport or incineration activities.

From the above it can be concluded that the protection of the environment in a comprehensive way is not guaranteed by simply using the TPH-approach (e.g. loss of soil fertility, production of new pollutants). Nevertheless, the statutory situation which is presently in force in Switzerland is still based on this approach. A summary of some relevant Swiss Federal regulations is presented in Table 6-1.
Since no specific regulations concerning the reuse of bioremediated soils exist, these regulations, initially introduced to control waste disposal, are adopted. However, by taking a closer look at these regulations, some irregularities can be identified between them:

i) the defined TPH concentrations are different in the two regulations regarding TPH in soils (VSBo-M4 [BUWAL, 1993] and AHR [BUWAL, *in preparation*]), and

ii) the suggested reuses for soils with tolerated residual contaminants therein are also very different. While the VSBo-M4 regulations allow to reuse the soil as top soils, e.g. on sport fields or in parks, the corresponding material, regulated by the AHR, cannot be reused unless it is sealed.

These differences can additionally cause confusion if the reuse of bioremediated soils has to be evaluated. Consequently, in order to assess potential reuses of bioremediated soils in a practicable but comprehensive manner as well as to answer the major question: *"when will the soil finally be remediated and therefore safe for a future reuse without any restrictions ?"*, an alternative approach should be defined.

Table 6-1 *Swiss federal regulations, already introduced [*)] or in preparation [**)]:*

contaminants	contaminants in soils				contaminants in soil eluates		
	VSBo-M4 [*) 1)]		AHR [**) 2)]		AltlV [*) 3)]	TVA [*) 4)]	
	Kat I [5)] [mg·kg^{-1}]	Kat II [5)] [mg·kg^{-1}]	"U" [6)] [mg·kg^{-1}]	"T" [7)] [mg·kg^{-1}]	C_{eluate} [mg·l^{-1}]	"I" [8)] [mg·l^{-1}]	"R" [9)] [mg·l^{-1}]
TPH	50	300			2	0.5	5
TPH (C_6-C_{10})			1	5			
TPH (>C_{10})			50	250			
Σ BTEX	0.7	7	1	5			
Benzene			0.1	0.5	0.01		
Ethylbenzene					3		
Toluene					7		
Xylenes					10		
Σ U.S.EPA-PAHs	1	5	1	15	i. reg. [10)]		

1) *VSBo-Mitteilungen; Nr.4 [BUWAL, 1993]*
2) *Aushubrichtlinie [BUWAL, in preparation]*
3) *Altlastenverordnung [BUWAL, 1998]*
4) *Technische Verordnung über Abfälle [TVA, 1996]*
5) *two categories for the reuse of contaminated soils at well defined locations*
6) *uncontaminated (unverschmutzter Aushub)*
7) *tolerated (tolerierter Aushub)*
8) *inert matter (Inertstoff)*
9) *residual matter (Reststoff)*
10) *individual regulations for each of the 16 U.S.EPA-PAHs*

6.1 How 'clean' is clean ?

The final goal of soil remediation activities must be the elimination of the environmental hazards due to the limitation of the emissions of potentially hazardous chemicals remaining in these soils. In order to determine the associated environmental hazards, the emissions of residual contaminants from bioremediated soils in addition to the total remaining TPH therein should be evaluated. Consequently, risk based emissions must be defined which could be tolerated in the environment and therefore accepted by the regulatory agencies. These acceptable emissions must be set conservatively low in order to prevent future contamination of any environmental media or any receptor potentially involved. A risk assessment for residual contaminants in bioremediated soils, including the potential relevant emissions, has been presented in the previous chapters.

As indicated in Chapter 2, the determination of contaminant concentrations in soil can be achieved by using different methods. Due to unequal sample preparations and extraction techniques, the recovery rates and the resulting soil concentrations can vary significantly, especially for contaminants with an elevated residence time in the soil. For example, a factor of 2.9 was determined between those methods evaluated in Chapter 2. From the data presented in the latest reports of the International Sediment Exchange for Tests on Organic Contaminants [SETOC, 1997a,b,c,d; 1998 a,b], a factor of 2 to 3 was determined for mineral oils in soil samples. With respect to the fact that tetrachloromethane is suspected to be carcinogenic [Römpp, 1992], this solvent should not further be used to extract mineral oil constituents from the soil. Consequently, an alternative solvent would be used with a reduced recovery rate for petroleum hydrocarbons. For example, 1,1,2-trichlorotrifluoroethane is widely accepted as solvent. It is, therefore, suggested that an additional safety factor of 3 should be introduced for the TPH concentrations in soil samples. Based on the risk calculations as well as this safety factor, the concentrations in Table 6-2 are suggested to be acceptable.

Table 6-2 *risk based, acceptable TPH concentrations/emissions from soils*

environmental compartment	exposure route	calculation based on	acceptable TPH concentrations
water	ingestion	RfD_o (n-hexane)	0.35 $mg \cdot L^{-1}$
ambient air	inhalation	RfD_i (n-hexane)	0.2 $mg \cdot m^{-3}$
soil	ingestion	RfD_o (n-hexane)	1.5 $g \cdot kg^{-1}$

The risk based approach must have been recognized by the working groups which presently are preparing future regulations on soil contamination. For example, formerly contaminated soils which still show elevated contaminant concentrations will be accepted for reuse, as long as the emissions of these contaminants could be prevented [BUWAL, 1998]. In contrast to the existing regulations regarding organic contaminants in soils [BUWAL, 1993], the future guideline on soil fertility [VBBo, 1998] does not regulate TPH in soils. It must have been recognized by the corresponding working group that mineral oil contaminants in soils can not be regulated by simply determining a surrogate parameter (such as TPH) in the soil. These contaminants have to be assessed individually on a case by case investigation. If regulations for specific contaminants are missing, however, the soil fertility must be guaranteed in the future [VBBo, 1998]. For the preservation of soil fertility: i) the biological communities in the soil as well as the plant growth and the plant quality must not be reduced and animals as well as humans must not be harmed [VBBo, 1998]. Unfortunately, no guidelines are included on how to determine the different parameters in order to define the soil fertility. It therefore is strongly recommended that such guidelines should be defined for the future. According to Gupta [1998], risk based threshold values can be adopted from other countries if the specific contaminants are not yet regulated by the VBBo.

In order to investigate and evaluate TPH contaminants in bioremediated soils in the future, an alternative procedure to the TPH-approach is presented in the following

paragraph. If other chemicals are present in this soil as well, however, they must be evaluated separately according to the corresponding regulations.

6.2 Suggested procedure to evaluate TPH contaminants in bio-remediated soils

A flowchart of the suggested procedure is presented in Figure 5-1. A threshold value of 1'500 $mg \cdot kg^{-1}$ is suggested for the residual TPH concentrations in bioremediated soils. If the TPH concentration in the soil is above this threshold value, the soil can not be reused without further examination since the calculated concentrations for soil ingestion is exceeded. Instead, the soil must either be further remediated, an alternative treatment technology must be used or only restricted reuse could be accepted (e.g. if the soil would be sealed and no human access would be possible). If the TPH concentrations are below 1'500 $mg \cdot kg^{-1}$ and no significant signals can be detected in the HRGC chromatograms, e.g. typical signals of fresh diesel fuels as presented in Figures 1-1 A and B, the TPH emissions from the soil must be determined. If none of the corresponding threshold values are exceeded (Table 6-2) and if the hazard index is below one, the investigated soil could be reused without any restrictions. As a prerequisite for all these evaluations and decisions, standardized sample preparations and analytical procedures must be employed to determine the TPH concentrations in water, air and soil samples.

A modified procedure could also be employed for soils which are suspected to be contaminated with mineral oil products in general.

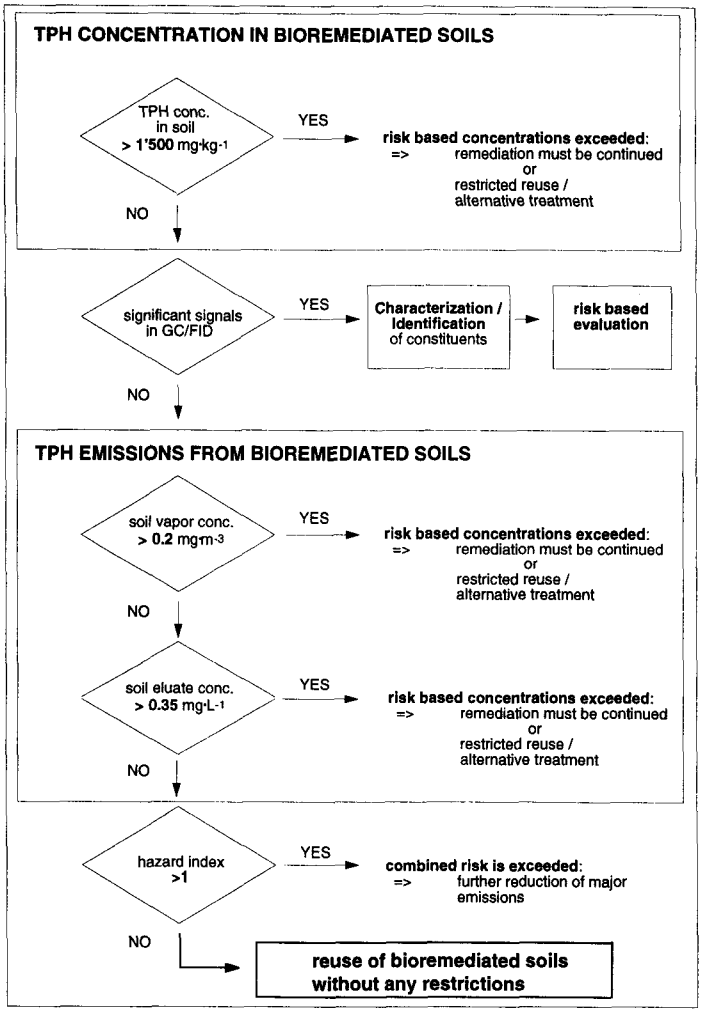

Figure 6-1 *Flowchart for the evaluation of the TPH contaminants in bioremediated soils*

6.3 Suggested procedure to evaluate TPH contaminants in mineral oil contaminated soils

Since a variety of different soils from different locations with varieties of contaminants therein must be screened, it is essential to include a fast and inexpensive method to identify potential environmental hazards. This is even more so if it is taken into consideration that the majority of the suspected and therefore investigated soils are recognized as not to be contaminated [EDI, 1997]. As a first step, the TPH concentration, besides other chemicals of concern, must be determined in a suspected soil sample as well as the background concentrations in uncontaminated reference samples from a nearby location. In contrast to existing regulations, the difference in the TPH concentrations of the two samples rather than the absolute TPH concentration in the contaminated sample must be evaluated, since elevated natural background concentrations (e.g., caused by natural organic material in soil, such as humic substances) could falsely be determined as contaminants [Gustafson, 1998]. A difference of 50 $mg \cdot kg^{-1}$, a concentration in soils which is widely accepted by existing regulations, could be adopted. This threshold value is set conservatively low in order to be protective for the environment. If this threshold value is exceeded, further investigations, mainly on the TPH emissions from the soils, are required as indicated in Figure 6-1. A modified flowchart of the suggested procedure is presented in Figure 6-2.

If the soils are accepted to be reused without any restrictions, they could also be used as top soil. For top soils, however, the regulations on contaminants in soils [VBBo, 1998] must be met. It therefore is inevitable to define the procedures which are necessary to determine the preservation of soil fertility. Nevertheless, based on the results obtained from the risk assessment presented in the previous chapters, it is indicated that the soil fertility would not be reduced by the residual contaminant in the bioremediated soil investigated in this study.

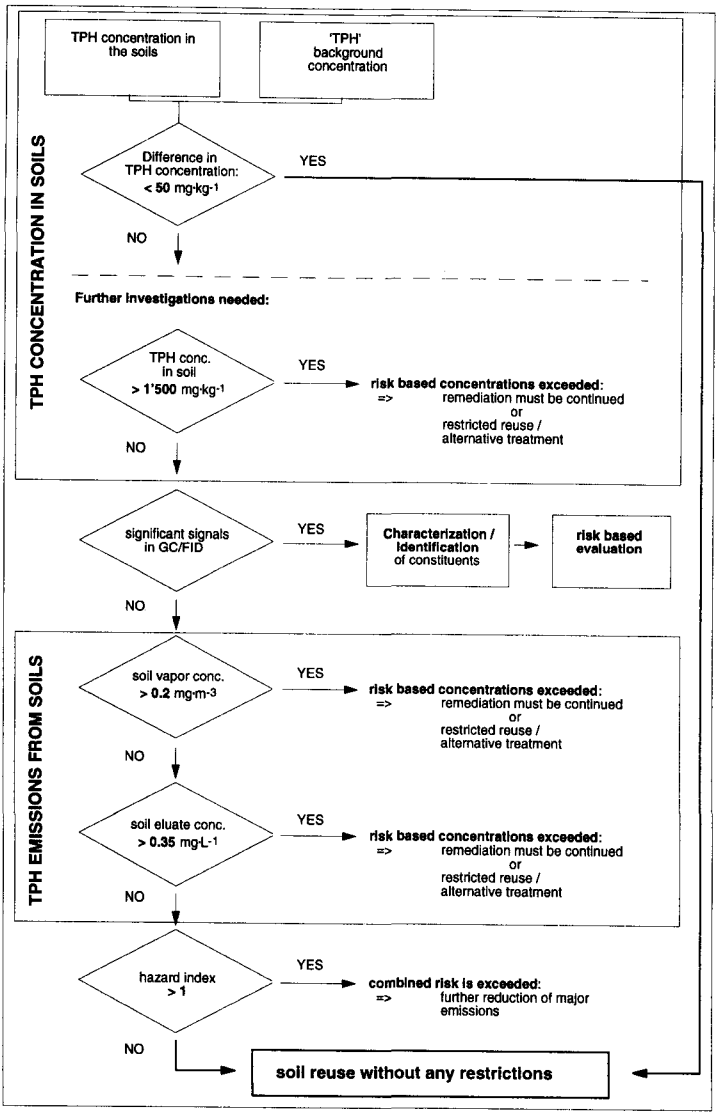

Figure 6-2 *Flowchart for the evaluation of the TPH contaminants in mineral oil contaminated soils*

6.4 Consequences for authorities

In Switzerland, soils with elevated mineral oil contaminant concentrations, caused by anthropogenic activities, is a topic which is presently widely discussed. The regulations presently employed in this country suggest that mineral oil contaminated soil should be assessed on the basis of the TPH concentration in the solid matter [BUWAL, 1993]. As discussed above, however, this is unsatisfactory. Instead, the emissions of the soil contaminants should be employed in the future as suggested by the alternative procedures summarized in Figure 6-1 and 6-2. Since standardized threshold values could be simply compared with specific data measured in field or laboratory investigations, the suggested procedure is presenting a tool which is as easy to use as the TPH-approach. In addition, the potential environmental hazards could be identified very fast and consequently, the most appropriate alternative to treat this soil could be defined. Furthermore, the threshold values are risk based and therefore could be explained. Moreover, different cases could easily be compared with each other since the decisions would all be based on the same standardized procedures. If a soil could be identified not to cause any environmental concerns, that is if the environmentally hazardous constituents therein would have been eliminated due to the remediation activities, it would not be necessary to dispose it on a landfill. Therefore, less soil material would have to be listed in an inventory for contaminated sites [BUWAL, 1998] and consequently, less sites would have to be monitored in the future and therefore less responsibilities would be passed on to the following generations.

6.5 Consequences for remediation companies

Standardized situations would be created by adopting the suggested procedure for all decision processes where mineral oil contaminated soils are involved. From the beginning, the remediation goals would be strictly set and must not be negotiated. A solid basis would be created for the remediation companies to evaluate whether or not a specific remediation technology could be successfully carried out. Further-

more, the same procedure could be employed to accompany the remediation activities, that is to identify the remediation success at any time of the treatment as well as to determine the endpoint of the remediation process. If the remediated soil could be reused without registering the future site in an inventory for contaminated sites, an alternative reuse other than the disposal on a landfill could be planned. This would be an important prerequisite to consider bioremediation as a remediation alternative, since no potential user would accept the disadvantage that a site would be disqualified because formerly contaminated soil was incorporated.

7

final discussion

Determination of residual contaminants

No significant concentrations of the commonly selected chemicals of concern in mineral oil products (Table 1-4) could be identified in the bioremediated soil investigated in this study. Nevertheless, as presented in Figure 2-3, a multitude of compounds remained which potentially could cause environmental concern. Compared to the data presented in Table 4-2 as well as Figure 4-1, however, components with very similar physicochemical properties at elevated concentrations could be detected in uncontaminated reference samples. Therefore, it appears that the residual contaminants from bioremediated soils as well as the components extracted from the uncontaminated reference samples could be very

much alike. Furthermore, the soxhlet extraction used for residual mineral oil constituents in this study is also including compounds which occur naturally in soils. To clearly assign elevated concentrations in soil as contaminants, it is inevitable to investigate the background concentration therein as well.

Biodegradation and bioavailability

Biodegradation is affecting the spreading of the residual contaminants from bioremediated soil in two ways. First, successful biodegradation of the contaminants is expected to result in a decrease of the dissolved fraction and second, dissolved contaminants which potentially are bioavailable can further be degraded (Table 3-2), leading to reduced environmental concentrations. However, as demonstrated in Chapter 3, the residual contaminants in bioremediated soil decreased only very slowly with increasing time. It was assumed that this was due to the highly limited bioavailability of the contaminants. It is reported, that part of the contaminants appear to be inaccessible for biodegradation, particularly in old contaminated sites [Bosma et al., 1997]. For example, very slow degradation was observed in a soil that was contaminated with PAHs even after inoculation with bacteria known to effectively degrade these compounds. After the addition of extracted and restored PAHs into the extracted soil material, rapid degradation of the contaminants was observed [Weissenfels et al., 1992]. Furthermore, treatments involving a rigorous mixing of the soil, breaking up the larger soil particles, stimulated biotransformation drastically [Doelman et al., 1990]. These observations indicate a reduced bioavailability of the contaminants which are present in the soil for a prolonged period of time. If contaminants are present in a remote micropore, have partitioned into some solid phase in the soil, or both, they are inaccessible to microorganisms, plants, and animals [Alexander, 1995]. It is reported that the mass transfer – and not the intrinsic microbial activity – is the critical factor in bioremediation in most cases [Bosma et al., 1997; Zhang et al., 1998]. It is a widely accepted fact that surfactants increase the solubility of many organic compounds. This physical remoteness of aged compounds and their diffusion to locations that

are biologically inaccessible are key considerations in assessing risk of contaminants in soil [Alexander, 1995]. Some contaminants or metabolites thereof can be linked to macromolecular organic matter (e.g., humic substances), a phenomenon which has major implication for the transport, toxicity and bioavailability of these compounds [Richnow et al., 1994]. In the literature it is reported that, in the context of bioremediation, the transformation of contaminants to irreversibly bound forms may be more of a solution than a problem [Zhang et al., 1998]. Burgos et al. [1996] reported that irreversible binding may become competitive to biodegradation. However, much more understanding is needed before engineered solutions should rely upon these mechanisms.

Toxicity tests

By following the procedure in preparing aqueous soil extracts (Chapter 4), constituents which could adversely affect the groundwater or surface waters are extracted from the soil. This extraction method simulates a worst-case scenario, since other parameters such as the amount of leachate, the nature of the subsoil layers and the flow pattern in the soil are not considered [Kreysa and Wiesner, 1995]. The procedure therefore could lead to concentrations in the eluates which would be above those found under natural conditions [Kreysa and Wiesner, 1995]. This can be confirmed by comparing the data in Figure 3-4 to that for the bioremediated soil *Pfyn* in Table 4-2. The concentration in the soil eluate is higher by a factor of 2 to 7 compared to that in the leachates.

Toxicity tests are not capable to provide a definite answer regarding the potential hazards associated with certain contaminants to the environment, simply because not all the species of concern can be identified and tested [La Grega, 1994]. Furthermore, by measuring the toxicity of contaminants to single organisms, the objective of *ecotoxicology*, that is the determination of the effects of contaminants on ecosystems [Moriarty, 1983], can not be achieved.

Toxicity calculations

As presented in Chapter 5, the toxicity potential for humans was calculated by using n-hexane as a worst case reference compound. One objection to these calculations could be that, instead of a representative of the PAH-like compound class (Table 2-1), a noncarcinogenic representative of the aliphatic-like compound class was used. Since PAHs very often are treated equivalent to the sixteen PAHs regulated by the U.S.EPA, the name of this fraction could be misleading. In fact, from the 12.1 % of the TSEM designated as PAH-like compounds, less than 3 % of this compound class were identified as one of the EPA-PAHs. The majority of the compounds detected in this fraction remained unidentified and subsequently, no specific statement on their toxicity could be made. Nevertheless, the concentrations of the EPA-PAHs in the bioremediated soil *Pfyn* investigated in this study were compared to the corresponding PRGs [U.S.EPA, 1996a] in residential soils. It was found that the PRGs were higher by a factor of 10 and more than the concentrations in the soil and therefore, humans, including sensitive groups, would be protected against these contaminants over a lifetime [U.S.EPA, 1996a]. However, this example indicates that the results presented in this study can not be adopted for other soils without a proper re-evaluation of each soil and the corresponding contamination therein.

Future actions needed

As a major request, the procedures and regulations which are employed for the evaluation of contaminants in the environment must be standardized. Therefore:

i) it is inevitable to standardize the sampling strategy, the sample preparation and storage as well as the analytical determinations of the contaminants within these samples. Based on this standardization, the results from different sites can be compared with each other and, more evident, these results can be compared with the corresponding threshold values in the various environmental compartments. A fast assessment of formerly contaminated soils would then be acceptable.

ii) as stated earlier, the future regulation concerning contaminants in soils (e.g., VBBo [1998]) and other environmental compartments must define both the criteria which have to be met as well as the procedures how to determine these criteria in the soil. This statement may sound trivial but it has not been adopted in all the regulations yet.

In order to complete the suggested procedures in Figures 6-1 and 6-2, the restricted reuses of soils which do not meet the criteria must be defined. For example, it is suggested that soils which show concentrations above the corresponding threshold values for either TPH concentrations in soil or soil vapor samples should not be used in residential areas or as top soils. Soils with elevated concentrations in the soil eluates should not be reused in groundwater protection areas. Furthermore, the monitoring of sites where a restricted reuse of bioremediated soil would be tolerated must additionally be regulated.

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