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# Relation between Bioavailability and Fuel Oil Hydrocarbon Composition in Contaminated Soils

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Bioavailability of oil components in contaminated soils is an important regulating factor for biodegradation rates. Changes in the composition of mineral oil can provide information regarding the bioavailability restrictions in contaminated soils. The fate of oil components was studied in a lysimeter experiment and laboratory incubations. A shift in the *n*-alkane ratios in the range *n*-C16:*n*-C20 was observed around 4.0 g kg<sup>-1</sup>, indicating that two different mechanisms control the bioavailability of the oil. At higher concentrations, the bioavailability was controlled by solubilization from a non-aqueous-phase liquid into the aqueous soil water phase. The ratios remained constant with decreasing oil concentration in this stage. Below 4.0 g kg<sup>-1</sup>, desorption and diffusion became rate-limiting factors: a shift was observed in the *n*-alkane ratios, showing that biodegradation rates of *n*-alkanes increased with decreasing carbon number. The monitoring of *n*-alkane ratios can be used to improve the efficiency of bioremediation treatments.

## Introduction

Bioavailability is considered a priority research objective in the bioremediation field (1), as it is an important but yet poorly quantified regulatory factor for the biodegradation of oil and other organic compounds in contaminated soils (2-5). An important but complex question for optimization of bioremediation processes is: When is bioavailability limiting the actual biodegradation rate? Besides bioavailability, biodegradation rates depend on (i) the presence of microbiota with the metabolic capacity to degrade the components of interest (6, 7); (ii) the recalcitrance of the compounds in the oil mixture (6, 8-12); and (iii) growth and activity factors (temperature, nutrients, electron acceptors, pH) influencing the microbial population dynamics (10, 13-17). In-situ and ex-situ bioremediation treatments can only be managed efficiently if the actual limiting factor for biodegradation rates is known. For example, the addition of nutrients and/or electron acceptors is not efficient when the biodegradation

rate is limited by the bioavailability of the contaminant. Modeling studies also show the need for the identification of this limiting factor (18, 19). Furthermore, the toxicity of organic compounds in the soil is strongly linked to bioavailability.

Bioavailability becomes more limiting as the oil concentration reduces from "high" to "low" because the absolute amount of available hydrocarbons decreases. However, kinetics of oil degradation in soil usually do not follow a first-order pattern: as oil concentrations decrease, the rates drop more than proportional to the concentration (8, 10, 12). Two mechanisms may be responsible for this deviation from first-order kinetics. First, preferential degradation of certain oil components causes a higher persistence of the remaining fraction, as was discussed in several studies (6, 8-12). Second, mass transfer limitations become important when the contaminant has been diffused to non-available sites. Currently, no other means than costly laboratory desorption experiments are available to identify these bioavailability restrictions.

The present work aims to identify a sensitive and easily accessible parameter for the identification of bioavailability restrictions in historically contaminated soils by monitoring the composition of the oil contaminant in the course of the degradation process and by relating relative degradation rates to bioavailability limitations. More specifically, the fate of *n*-alkanes in the oil mixture is discussed. The *n*-alkanes in oil are a class of components having a similar metabolic degradation pathway and a decreasing aqueous solubility with chain length. The results presented in this paper show that the *n*-alkane ratios contain information that can be used for the distinction of processes regulating the bioavailability of oil in the soil.

## Experimental Design and Analytical Methods

Samples from three type of experiments were analyzed to study the fate of oil components under various environmental conditions. First, dynamic field conditions were simulated in a lysimeter study (19). Second, laboratory batch incubations were conducted with non-saturated soil to study biodegradation under static conditions (20). Third, slurry phase batch incubations were carried out to study the influence of aggregate structure on the biodegradation rates (21). The latter will be referred to as "slurry phase" incubations to distinguish from the incubations with non-saturated soils. All experiments were carried out with the same soil, a contaminated silt loam that was sampled from an oil refinery site at Pernis, The Netherlands. The soil was sieved to remove coarse stones and homogenized before further use. Some relevant properties are as follows: clay 8.3%; silt 19.0%; CaCO<sub>3</sub> 90 g kg<sup>-1</sup>; pH<sub>H2O</sub> 7.85; pH<sub>CaCl2</sub> 7.42; organic matter 2.6%; total P, K, and N of 0.54, 12.7, and 0.71 g kg<sup>-1</sup>, respectively.

Semi-field scale (0.9 × 0.9 × 0.9 m) lysimeter experiments were carried out in a controlled greenhouse environment over a 20-month period. The lysimeters were constructed of concrete and insulated with polystyrene foam plates, and a drain was installed in a 0.30-m sand layer. The lysimeter experiment started at March 30, 1992, by packing a layer of 0.6 m of the homogenized contaminated soil on top of the drain sand, with a dry bulk density of 1506 kg m<sup>-3</sup>. The initial oil content of the lysimeters was nearly homogeneous: 13.2 ± 1.0 g kg<sup>-1</sup> (*n* = 5, measured by a commercial laboratory). Only non-composite "point" samples will be considered in this paper for the analysis of the oil composition. The oil concentration and oil composition of these samples was measured as described later in this section. Data are presented from two lysimeters, of which 32 point samples

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were taken (300 cm<sup>3</sup>) at three depths of 5, 35, and 55 cm. The amount of sampling events was limited to minimize the disturbances of the soil structure. In lysimeter A, samples were taken in the second year, at day 34, 97, 181, 250, and 331. In lysimeter B, samples were taken in the first year at day 196, 231, and 308 and in the second year of the experiment at day 615 and 706.

In lysimeter A, the lower boundary temperature of the soil could be controlled at 35 °C using a heating cable. The lysimeters were aerated in the second year of the experiment, starting at day 34, by extracting air from the soil through the drain with a high-capacity air pump. The lysimeter temperature, CO<sub>2</sub> efflux, CO<sub>2</sub> and O<sub>2</sub> concentrations, water content, and pressure head were monitored throughout the whole measuring period (19). The air temperature in the greenhouse varied between 2 and 45 °C due to the annual temperature cycle. The water content was between 0.05 and 0.27 g g<sup>-1</sup> with an average water content of 0.16 g g<sup>-1</sup>.

Subsamples with a TEHC concentration of 6.7 g g<sup>-1</sup> were taken from lysimeter B for use in laboratory incubations with unsaturated soil. These incubations were carried out with 70-g samples in closed jars (400 cm<sup>3</sup>) equipped with a septum (20). Three duplicate treatments were incubated at a temperature of 20 °C with a water content 0.20, 0.17, and 0.14 g g<sup>-1</sup>, respectively. The incubation time in the laboratory was up to 240 days, and the headspace was reopened every 30 days to allow gas exchange (20). The total extractable hydrocarbon (TEHC) concentration was measured at the end of the incubation.

Similar incubations were carried out with soils in the slurry phase (21). Subsamples with a TEHC concentration of 3.0 g kg<sup>-1</sup> were taken from lysimeter A. A total of 10 g of soil was mixed with 50 mL of aqueous solution and incubated at 25 °C on a rotary shaker (90 movements min<sup>-1</sup>). Three treatments were incubated in triplicate, with an aqueous solution containing 10 mM CaCl<sub>2</sub>, 10 mM NaCl, and a control containing 15 mM NaN<sub>3</sub>, respectively (21). The total incubation time for these experiments was 25 days, and the headspace was not ventilated as the oxygen concentrations did not reduce below 10% (v/v).

Extractions for TEHC determination were performed in triplicate with 20-g subsamples, except the samples incubated in the slurry phase which were completely used for TEHC extractions. The soil was extracted twice with an acetone-pentane mixture (1:2 v/v, 30-min shaking of extraction) using octane (*n*-C<sub>8</sub>, Aldrich, 29,698-8) as a recovery standard. Acetone was removed in a separation funnel, and Florisil (Merck 12518) was added to remove extracted humic polar compounds. An internal standard (*n*-C<sub>36</sub> or *n*-C<sub>38</sub>) was added to the extract. The hydrocarbon concentration in the extract was quantified by gas chromatography (DB-1 capillary column, 60 °C, 4 °C/min to 320 °C, flame ionization detection). The internal standards were baseline resolved because the extract contained hydrocarbons in the range of C<sub>10</sub>–C<sub>35</sub>. A blank sample (hexane) was run after every third injection to avoid column bleeding. The extraction recovery was 89 ± 10%, and the average standard deviation of three replicate extractions from the same sample was 10%. The injection reproducibility was better than 2%.

The TEHC concentration was calculated by group integration (C<sub>10</sub>–C<sub>35</sub>) of the resolved and non-resolved peaks after subtraction of a blank run. The internal standard response factor (ISRF) of mineral oil was measured by running a quantitative reference sample, obtained from the Dutch National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands). An ISRF of 1.12 was used for the calculation of the absolute TEHC concentration. The *n*-alkanes were quantified by valley-to-valley integration and were identified by running an *n*-alkane standard mix (Supelco D2887). Pristane (2,6,10,14-tetramethylpentadecane, C<sub>19</sub>H<sub>40</sub>) and phytane (2,6,10,14-tetramethylhexadecane, C<sub>20</sub>H<sub>42</sub>) were identified by gas chromatography–mass spectroscopy). The detection limit for alkanes was 0.0008 g kg<sup>-1</sup>. The concentrations of compounds lower than *n*-C<sub>11</sub> were at non-detectable levels.

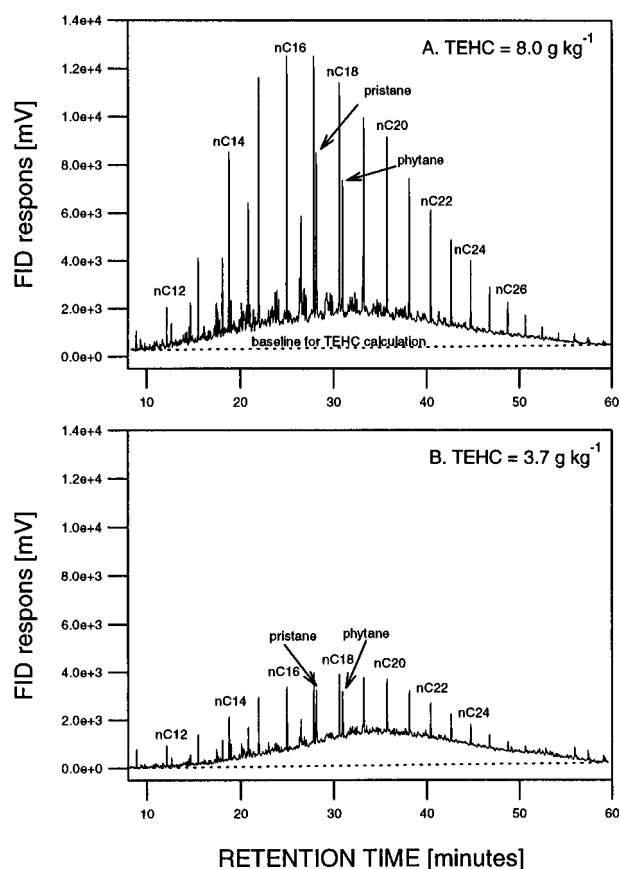


FIGURE 1. Gas chromatogram at high (A) and intermediate (B) TEHC concentrations. The *n*-alkanes are preferentially degraded over the “hump” that is formed by unresolved branched alkanes.

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## Results and Discussion

**Relative Degradation Rates of Oil Compounds.** The decrease of the oil concentration in the contaminated soil was due to biomineralization, as was evidenced by respiration measurements in the lysimeters and the laboratory incubations (19–21). A well-known phenomenon from biodegradation of oil is the relatively fast disappearance of the *n*-alkanes in comparison with the other compounds (8, 9, 11). The chromatograms in Figure 1 illustrate that the *n*-alkanes disappear faster than the unresolved hump, mainly consisting of branched alkanes. Branched alkanes are more resistant to microbial degradation than *n*-alkanes due to their molecular structure (6). It has to be noted that the “initial” situation is not a freshly spiked oil, because the soil was historically contaminated. Thus, Figure 1A shows a chromatogram of an oil extract that is already strongly weathered. The chromatographic conditions were optimal because the *n*-alkanes in the non-weathered standard mineral oil were more than 97% resolved.

The preferential degradation of *n*-alkanes becomes manifest by observing a time series of the TEHC concentrations and *n*-alkanes at a specific location. This is illustrated in Figure 2 for lysimeter A at two different depths, 5 and 35 cm. Note that the TEHC concentrations at day 34 is 3.5 and 6.7 g kg<sup>-1</sup> for the 5 and 35 cm depth, respectively. This oil concentration gradient with depth was due to higher biodegradation rates in the surface layer during the first year of the experiment, as a result of decreasing oxygen availability with depth (19). Lysimeter A was ventilated in the period

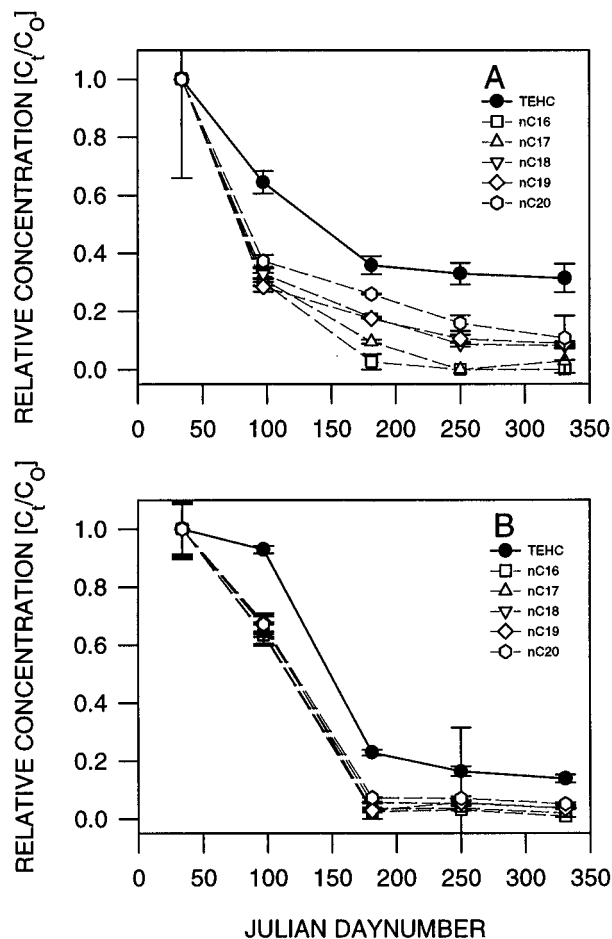


FIGURE 2. Time series of relative hydrocarbon concentrations in lysimeter A at 5 cm depth (A) and 35 cm depth (B), in the second year of the lysimeter experiment. The TEHC concentration decreased in this period from 3.5 to 1.1 g kg<sup>-1</sup> (A) and from 6.7 to 0.9 g kg<sup>-1</sup> (B). The *n*-alkanes are preferentially degraded, and the residual concentrations increase with the carbon number. The standard deviations refer to the analytical quantification error.

shown in Figure 2, and therefore the vertical concentration gradient was reduced during the measurement period shown in Figure 2.

The relative concentrations used in Figure 2 allow a comparison of TEHC degradation with the disappearance of *n*-alkanes. The range *n*-C16–*n*-C20 was chosen because these alkanes are non-volatile and relatively abundant in the oil mixture (see Figure 1). Therefore, it can be accurately quantified, and it may be safely assumed that biodegradation was the primary mechanism of disappearance. Figure 2 confirms that the *n*-alkanes were degraded more rapidly than the TEHC fraction. The relative TEHC concentration at 5 cm depth is decreased to 0.3 at the end of the period, whereas relative concentrations of the *n*-alkanes are all below 0.15. The residual concentrations of the *n*-alkanes decrease with increasing carbon number, reflecting that the chain length of the *n*-alkanes influenced the biodegradation rates. Similar trends are observed in Figure 2B.

**Analysis of Ratios of Oil Components.** The analysis of time series does not allow for a direct comparison of all the data from the lysimeter and the incubation experiments. Instead, relative disappearance rates can be analyzed by observing the ratios of different oil components as a function of TEHC concentration, as is illustrated in Figures 3 and 4. The time dimension is thereby eliminated, and the data with different biodegradation rates can be directly compared. For example, if the component ratio A:B decreases when going from high to low TEHC concentrations, this reflects that

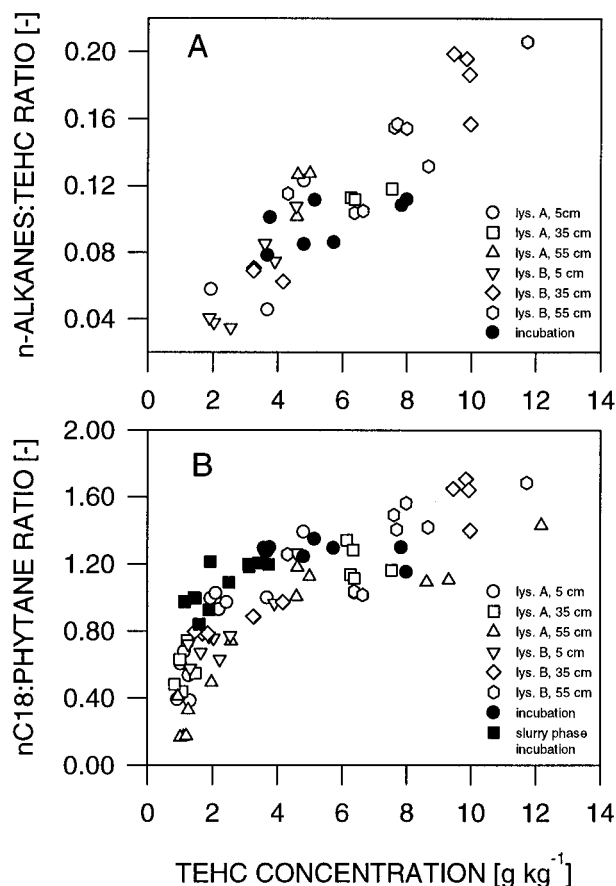


FIGURE 3. Ratio of *n*-alkanes over the unresolved fraction (A) and the *n*-C18:phytane ratio (B) as a function of TEHC concentration. The relation confirms the preferential degradation of the *n*-alkanes as shown in Figure 1 and 2.

component A is degraded at a higher rate than component B. The use of ratios allows for a quantitative comparison even if *n*-alkanes are not baseline resolved. It should be noted that the concentration differences in Figures 3 and 4 are due to biodegradation of the oil because the soil was initially homogenized before the lysimeters were filled.

The results from lysimeter experiments and incubations with water unsaturated soil are discussed first. The first approach was to analyze the influence of biodegradability, the molecular recalcitrance to biomineralization, by using ratios of compounds with a similar aqueous solubility but a different molecular structure (Figure 3). Second, the influence of bioavailability is studied by plotting *n*-alkane ratios against TEHC (Figure 4). The *n*-alkanes are a class of components having similar metabolic pathways but a different aqueous solubility. This second approach is new and can be related to bioavailability of oil components in the soil.

The ratio of the resolved *n*-alkanes over the total TEHC concentration decreases with decreasing TEHC concentration (Figure 3A), confirming the preferential consumption of the *n*-alkanes over the branched alkanes that was illustrated in Figures 1 and 2. This ratio could only be calculated down to a TEHC of 2.0 g kg<sup>-1</sup> because the fraction total *n*-alkanes could not be quantified below this concentration. The higher *n*-alkanes were present in much smaller amounts than in the range *n*-C16–*n*-C20 (see Figure 1), and the integration area was under the detection limit at the lower TEHC concentrations. The relationship in Figure 3A is linear, reflecting that the microbial preference for *n*-alkanes is constant regardless of the amount of hydrocarbons present in the soil. A similar trend is obtained for the *n*-C18:phytane ratio (Figure 3B). This ratio has been used as a qualitative measure of biodegradation (22) as phytane is a relatively persistent

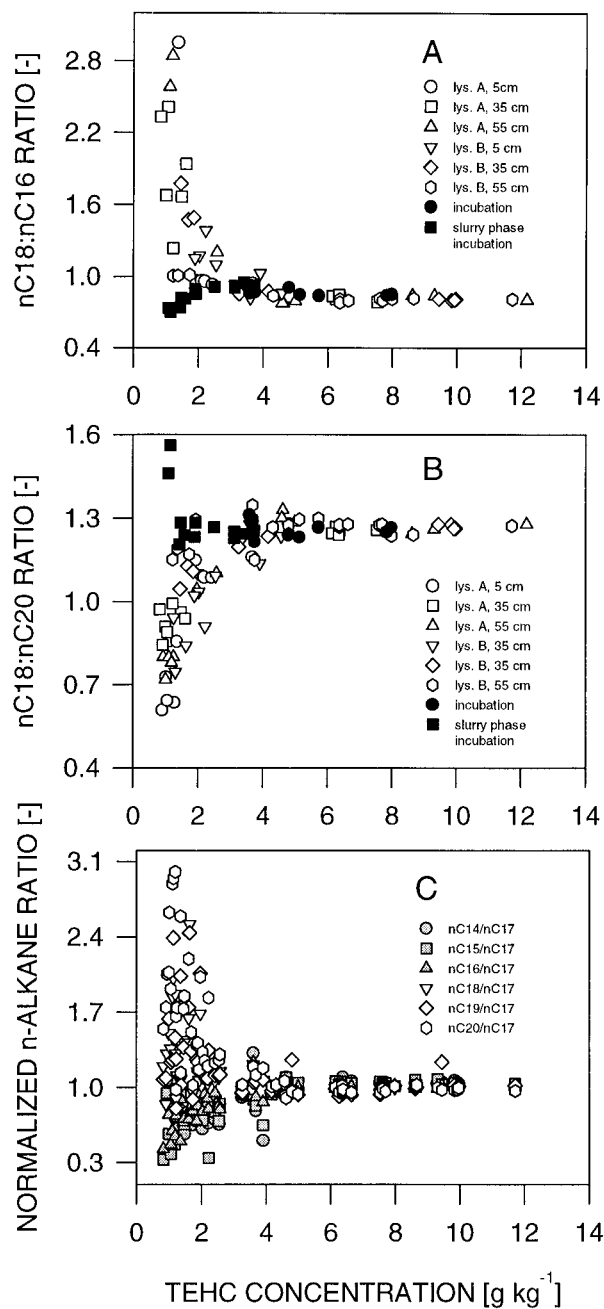


FIGURE 4. *n*-Alkane ratios as a function of TEHC concentration for lysimeter and laboratory incubation experiments (A and B). Panel C shows only data from the lysimeter experiments; these ratios are normalized for their averaged value in the concentration range of 12–4 g kg<sup>-1</sup>. In the unsaturated soil, the ratios are constant between 12 and 4 g kg<sup>-1</sup>, but below 4 g kg<sup>-1</sup>, the *n*-alkanes with a lower carbon number are preferentially degraded.

isomer. In this contaminated soil, phytane is also biodegraded, although at a slower rate than *n*-C18. However, the relation between TEHC and *n*-C18:phytane appears to be convex, implying that at lower concentrations the preferential degradation of *n*-C18 over phytane is more pronounced. A possible explanation for this will be given in the next section.

An important result is obtained when plotting the *n*-C18:*n*-C16 and *n*-C18:*n*-C20 ratio as a function of TEHC concentration (Figure 4A,B). At higher TEHC concentrations (>4.0 g kg<sup>-1</sup>), the ratios remain constant. Apparently, all *n*-alkanes are degraded at the same rate, regardless of the chain length of the *n*-alkanes. A shift in the *n*-C18:*n*-C16 (increasing) and *n*-C18:*n*-C20 ratio (decreasing) is observed as TEHC concentrations decrease below approximately 4.0

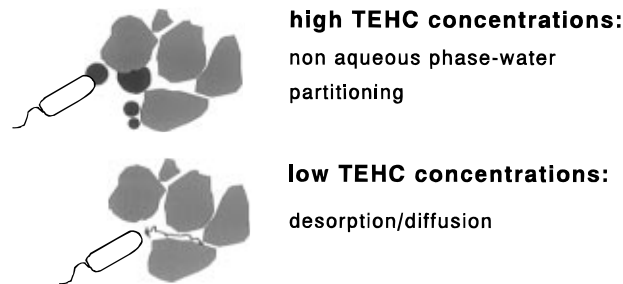


FIGURE 5. Schematic model of different mechanisms regulating the bioavailability in oil-contaminated soils. At higher concentrations, the bioavailability is regulated by solubilization from a NAPL phase. At lower concentrations, the bioavailability is regulated by desorption and diffusion processes.

g kg<sup>-1</sup>. Note that this is only the case for the water unsaturated samples. Similar patterns can be observed for other *n*-alkanes, becoming more pronounced as the difference in carbon number increases (Figure 4C). Note that in Figure 4C the ratios are normalized for their average value in the concentration range 3–12 g kg<sup>-1</sup>. This is necessary to compare the different ratios, because the initial concentration of the *n*-alkanes in oil mixtures is not the same for different *n*-alkanes.

The ratios could be affected by analytical errors, especially at lower concentrations. However, the observed shifts in the ratios are most likely not caused by such errors as the shifts start at a TEHC concentration at which the integration areas for the *n*-alkanes are 10–30 times higher than the detection limit. Furthermore, errors in the ratios are expected to be random and not systematic: a ratio is affected by the integration areas of two peaks that are quantified by the same algorithm and are close together in the chromatogram. Any systematic error in the integration would equally apply for both the peak areas and would not be manifest for the ratio of the two areas.

It is quite surprising that the trends in Figures 3 and 4 become evident despite the differences in the temperature, water content, and aeration of the different lysimeter and (non-slurry) incubation conditions. Results from different experiments are integrated in Figures 3 and 4, and no correlations between these environmental factors and the *n*-alkane ratios were observed. However, the ratios of the slurry samples clearly deviate in Figure 4A,B, which will be discussed in the next section.

***n*-Alkane Ratios and Bioavailability.** Figure 4 shows that there is no influence of the chain length on the biodegradation rates of *n*-alkanes at higher TEHC concentrations (12.0–4.0 g kg<sup>-1</sup>). At lower concentrations (<4.0 g kg<sup>-1</sup>), components with a higher aqueous solubility are mineralized at a higher rate. This indicates that a different mechanism controls the uptake of the compounds by bacteria at lower concentrations. Figure 5 shows a schematic model of bioavailability, offering a plausible explanation for the shift in the *n*-alkane ratios. At higher concentrations, the oil is mainly present as a non-aqueous-phase liquid (NAPL), either as a film layer on soil particles or as dispersed droplets. The bacteria are present near the NAPL–water interfaces, and bioavailability is controlled by NAPL–water solubilization. Bacteria are able to degrade hydrocarbons at a rate higher than permitted by the water solubility, probably as a result of the production of biosurfactants (23). Hence, it is expected that the bioavailability is not importantly influenced by the aqueous solubility of the compounds in a oil mixture when the TEHC concentrations are high.

At lower concentrations, the contaminant is no longer present as a NAPL. The majority of the hydrophobic molecules are adsorbed to the soil matrix in this stage. Regardless of the sorption mechanism, molecules become

available after a desorption step followed by mass transfer to microbial cells. In this stage, the availability of contaminants is much lower due to diffusion processes. The aqueous solubility is expected to have a large impact on the diffusion rates and on the biodegradation rates. This could also explain the convex shape of the *n*-C18:phytane ratio, because diffusion rates of *n*-C18 (C<sub>18</sub>H<sub>38</sub>) are expected to be higher than phytane (C<sub>20</sub>H<sub>42</sub>).

Slurry phase incubations were used to confirm this interpretation. Samples were incubated in the slurry phase having TEHC concentrations of 3.0 g kg<sup>-1</sup>, because the diffusion limitation is most pronounced below this concentration. Mineral-organic matter aggregates are more dispersed in the slurry phase than in the unsaturated soils. Moreover, it was shown that the degree of aggregate dispersion correlated with biodegradation rates (21). Therefore, the influence of aqueous solubility should be less pronounced in the slurry phase. Indeed, the *n*-alkane ratios of the slurry phase samples considerably deviate from the other samples (Figure 4A,B). The ratios remain nearly constant below 3.0 g kg<sup>-1</sup>, or even show an inverse pattern as compared to the other samples. This fits in the schematic model of Figure 5 because a reduction in the diffusion distance, as a result of particle dispersion and mixing in a slurry, is expected to lead to equal biodegradation rates of the different *n*-alkanes.

#### Practical Implications for Bioremediation Treatments.

By using the above-described analysis, the *n*-alkane ratios derived from high-resolution gas chromatography provide information that could be used to optimize the management of in-situ and ex-situ bioremediation treatments. The bioavailability of the oil is controlled by NAPL-water solubilization as long as *n*-alkane ratios remain approximately constant. In an ex-situ application, bioavailability could be increased by tillage to facilitate a uniform distribution of the contaminant. Also, surfactants could be used to increase the solubility of the NAPL. However, other factors are possibly limiting in this phase. Our lysimeter studies revealed that oxygen was far more limiting than bioavailability at high TEHC concentrations (19). Therefore, management measures like aeration and fertilization can be very efficient during this stage.

A shift in the *n*-alkane ratios indicates that the bioavailability is primarily controlled by desorption/diffusion processes, and bioavailability becomes an important limiting factor. Tillage is no longer effective because the microstructures that are responsible for diffusion limitations are not affected by tillage. Furthermore, surfactants are less effective in this stage due to longer diffusion distances. For the data presented here, the observed shift occurs at a TEHC concentration of about 4.0 g kg<sup>-1</sup>. Respiration studies of the same soil revealed a similar shift in the mineralization coefficient in the same TEHC concentration range (19). This indicates that the microbial activity changed and possibly that other carbon sources become important at low TEHC

concentrations. However, the critical TEHC concentration where the shift occurs is dependent on the soil properties (soil structure, organic matter content) and the contamination history. A larger fraction of oil has been diffused to non-available sites if the soil is exposed to the contaminant for a longer time period. This effect is known as aging of the contaminant (24). At low TEHC concentrations, it is effective to increase bioavailability, whereas the other management options are expected to have only a limited effect.

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