

The Use of Sequential Supercritical Fluid Extraction for Bioavailability Investigations of PAH in Soil

A. P. Loibner, M. Holzer, M. Gartner, O. H. J. Szolar and R. Braun

Einsatz von Sequentieller Superkritischer Fluid Extraktion zur Untersuchung der Bioverfügbarkeit polyzyklischer aromatischer Kohlenwasserstoffe

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants that predominantly occur at manufactured gas plant sites, steel works and wood preserving stations, but they also occur as diffuse pollution over large areas of land at lower concentrations. They are high risk contaminants as some of them are proved to cause cancer or are at least under suspect to be carcinogenic (CAVALIERI and ROGAN, 1992; SHAW and CONNELL, 1994).

Thus, over the last decades several innovative remediation techniques have been developed and applied to PAH contaminated sites with different successes. Among these, various bioremediation efforts have shown promising results for the clean-up of soils contaminated with organic pollu-

tants (WINNINGHAM et al., 1999; CONOLLY et al., 1999). In spite of this, long remediation periods as well as high residual pollutant concentrations particularly for high-ring PAH have restricted such clean-up measures to a few full-scale applications only. Contaminant sequestration and inherent recalcitrance have been discussed to be responsible for a limited biodegradation of organic pollutants in soil (HUESEMANN, 1997). However, in the last years many authors have demonstrated considerable biodegradation even for high molecular weight PAH under optimized conditions in the absence of soil or soil like matrices (SMITH, 1990; BOUCHEZ et al., 1995; WARSHAWSKY, 1995; LI et al., 1996; SCHNEIDER et al., 1996; YE et al., 1996). Thus, various studies have focused on the sequestration of contaminants in soil as the major obstacle for microbial attack. The processes involved

Zusammenfassung

Der Einfluß des Gehaltes an organischer Substanz im Boden (SOM) auf die Bioverfügbarkeit von Polyzyklischen Aromatischen Kohlenwasserstoffen (PAH) wurde untersucht. Dazu wurden mikrobielle Abbauxperimente durchgeführt und die Ergebnisse mit den Daten einer Sequentiellen Superkritischen Fluid Extraktion (SSFE) verglichen. Zwei Modellböden mit unterschiedlichem Gehalt an organischer Substanz (Boden 1: 2.1 %, Boden 2: 8 %) wurden mit Anthracenöl kontaminiert, welches Fluoranthen als Hauptkomponente enthält. Nach der Kontamination wurden die Böden über 135 Tage gelagert, um den Einfluß der Alterung auf die biologische Abbaubarkeit sowie die Extrahierbarkeit von Fluoranthen zu bestimmen. Weiters wurden Experimente mit einem Industrieboden durchgeführt, der bereits vor Jahrzehnten kontaminiert wurde. Für nicht gealterte Proben wurde verstärkter Abbau in Boden 2 (höherer Gehalt an organischer Substanz) festgestellt. Für gealterte Proben jedoch, war der mikrobielle Abbau in Boden 2 geringer, was auf stärkere Sorption von Fluoranthen in humusreichen Böden zurückzuführen sein dürfte. Zur Beurteilung des Rückhaltevermögens bzw. Freisetzungsverhaltens von PAH im Boden wurde eine SSFE-Methode, bestehend aus acht aufeinanderfolgenden Extraktionsschritten, entwickelt. Für gealterte kontaminierte Modellböden wurden geringe Extraktionsausbeuten bei hohen Gehalten an organischer Substanz festgestellt. Diese Daten stimmen mit den Ergebnissen der mikrobiellen Abbauxperimente überein. Jedoch konnten für nicht gealterte Proben mit verschiedenen Humusgehalten keine unterschiedlichen Extraktionsausbeuten erzielt werden. Für Industrieboden wurde ein umgekehrt proportionaler Zusammenhang zwischen der Extraktionsausbeute und dem Molekulargewicht der PAH beobachtet.

Schlagnworte: PAH, PAK, Fluoranthen, mikrobieller Schadstoffabbau, Bioverfügbarkeit, Boden, Superkritische Fluid Extraktion, organische Substanz im Boden, Humus.

Summary

The influence of soil organic matter (SOM) content on the bioavailability of polycyclic aromatic hydrocarbons (PAH) was investigated by performing biodegradation experiments and comparing these data to results from a sequential supercritical fluid extraction (SSFE) method. Two model soils with different SOM (soil 1: 2.1 %, soil 2: 8 %) were contaminated using anthracene oil containing fluoranthene as the major high molecular weight PAH. After spiking, soils were stored for 135 days in order to assess the influence of aging on the biodegradation and extractability of fluoranthene. Moreover, experiments were also performed on an industrial soil, which was already contaminated several decades ago. For non-aged samples, advanced degradation was achieved in soil 2 (higher in SOM). However, for aged samples, degradation was lower in soil 2 indicating that sequestration was more pronounced in the high organic matter soil. To evaluate the retention/release behavior of PAH in soil as an important factor influencing their bioavailability, a SSFE method was developed comprising eight subsequent extraction steps. For aged spiked soils extraction data indicate a reduced recovery when high in SOM. These findings are in line with the respective microbial degradation results. However, for non-aged samples no difference in extraction recovery could be observed for both, low and high organic matter soils. Finally, for industrially contaminated soil an inverse relationship between the molecular weight of PAH and their extraction recovery was observed.

Key words: PAH, fluoranthene, biodegradation, bioavailability, soil, supercritical fluid extraction, soil organic matter.

are described to be diffusion into nanopores and sorption (adsorption and partitioning) to organic matter (PIGNATELLO and XING, 1996; XING and PIGNATELLO, 1997; LUTHY et al., 1997; NAM and ALEXANDER, 1998; GILLETTE et al., 1999). In-depth studies investigating phenomena such as aging, soil organic matter content (SOM), competitive sorption, pollutant concentration, water content, and temperature, etc. which affect sequestration were carried out by WEISSENFELS et al. (1992), CHUNG and ALEXANDER (1998), CHUNG and ALEXANDER (1999), HATZINGER and ALEXANDER (1997), NAM et al. (1998), XING and PIGNATELLO (1998), WHITE et al. (1998a), WHITE et al. (1998b) and BONTEN et al. (1999). In addition, the microbial formation of bound residues of PAH may change the bioavailability of the pollutants in soil. However, bound residues (as described by ESCHENBACH et al., 1998) are already a consequence of microbial attack and their production requires bioavailable contaminants. Therefore, the current work is focussing on the bioavailability of native PAH, exclusively.

Various methods, which include desorption (BONTEN et al., 1999), persulfate oxidation (CYPERS et al., 1999), application of surfactants (VOLKERING et al., 1998), extraction using alcohols or alcohol-water mixtures (TANG et al., 1999) as well as supercritical carbon dioxide (LOIBNER et al., 1997; LOIBNER et al., 1998) have been developed to determine the extent of sequestration and thus allowing an estimate of the bioavailable fraction of organic contaminants in soil.

The present study describes a novel and rapid sequential supercritical fluid extraction (SSFE) method which allows the assessment of the bioavailability of PAH in soil. The developed method was applied to two spiked (non-aged and aged) and one industrial soil from a former manufactured gas plant site. Supercritical fluid extraction data were finally compared to biodegradation results from microcosm experiments.

2. Materials and methods

2.1 Preparation of Soils

Initial experiments for the development of the SSFE method have been conducted using two model soils (soil 1, soil 2) different in organic matter content and texture (Table 1). Soils have been spiked with anthracene oil (VOEST Alpine Stahl; Linz, Austria) to achieve a total PAH concentration of 500 mg/kg dry soil. The main compounds present in anthracene oil are given in Table 2, all other US-EPA PAH are present in trace amounts only. Prior to spiking, soil samples have been sterilized using a tyndallization procedure (1h autoclaving at 100°C followed by 24 h incubation at 37°C, 3 repetitions). For spiking, anthracene oil was dissolved in dichloromethane (p.a., J.T. Baker; Deventer, Holland) and added to soil at a ratio of 1:6 (mL per g dry soil). After thorough mixing, the solvent was evaporated in a sterile laminar flow box over night. Aging was

Table 1: Parameters of model and industrial soils
Tabelle 1: Bodenparameter für Modell- und Industrieböden

Parameter / Soil	Model Soils		Industrial Soil Soil A
	Soil 1	Soil 2	
Type	Sandy Loam	Silty Loam	Silty Sand
SOM (%)	2.1	8.0	3.1
pH	7.5	7.6	7.1
Total PAH (mg/kg) ^(a)	500	500	368

^(a) Calculated from eight main PAH compounds, details are given in Table 2.

Table 2: PAH concentrations (major compounds) of anthracene oil used for spiking of model soils

Tabelle 2: PAH-Konzentrationen (Hauptkomponenten) von Anthracenöl, welches den Modellböden zugesetzt wurde

Compound	PAH Concentration (mg/g)	Abundance (%)
Naphthalene (NAP)	60.0	13.2
Acenaphthene (ACN)	45.5	10.0
Fluorene (FLU)	47.3	10.4
Phenanthrene (PHE)	142	31.3
Fluoranthene (FLT)	92.4	20.3
Pyrene (PYR)	52.9	11.6
Benz[a]anthracene (B[a]A)	9.3	2.0
Chrysene (CHR)	5.6	1.2
Total PAH	455	100

accomplished in teflon sealed glass vessels covered with aluminum foil at 4° C for 135 days.

In addition, an industrial soil (soil A) obtained from a former manufactured gas plant site was used for degradation and extraction experiments. Soil parameters and PAH concentrations are given in Table 1 and Table 3, respectively. The age of the spill is not exactly known, however, it can be assumed that the contamination of the soil occurred several dozens of years ago.

2.2 Biodegradation experiment

Sterilized and spiked soils as well as samples from the former manufactured gas plant site have been inoculated using an enrichment culture obtained from activated sludge cultivated on anthracene oil as single carbon source. Nutrients in form of $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (p.a., Merck; Darmstadt, Germany) and NH_4NO_3 (p.a., LOBA Feinchemie; Fischamend, Austria) were added sufficiently at a ratio of C:N:P = 100:5:1. Amounts of nutrients added were calculated from the carbon content of the soil with the highest organic matter content (soil 2). Water content was adjusted to

Table 3: PAH concentrations of industrial soil (soil A)
Tabelle 3: PAH-Konzentrationen des Industriebodens (soil A)

Compound	PAH Concentration (mg/kg)	Abundance (%)
Naphthalene (NAP)	2.6	0.7
Acenaphthylene (ACY)	5.3	1.4
Acenaphthene (ACN)	4.1	1.1
Fluorene (FLU)	14.2	3.9
Phenanthrene (PHE)	30.3	8.2
Anthracene (ANT)	32.2	8.8
Fluoranthene (FLT)	80.2	21.8
Pyrene (PYR)	61.7	16.8
Benz[a]anthracene (B[a]A)	32.6	8.9
Chrysene (CHR)	23.9	6.5
Benzo[b]fluoranthene (B[b]F)	25.7	7.0
Benzo[k]fluoranthene (B[k]F)	12.4	3.4
Benzo[a]pyrene (B[a]P)	23.7	6.4
Ideno[1,2,3-c,d]pyrene (I[1,2,3]P)	11.5	3.1
Dibenz[a,h]anthracene (D[a,h]A)	7.7	2.1
Benzo[g,h,i]perylene (B[g,h,i]P)	0.03	0.01
Total PAH	368	100

60 % of the maximum water holding capacity and soil samples (60 g DM) were incubated in glass beakers covered with aluminum foil at 20° C. For aeration, the soil was stirred every second day and consumed and evaporated water was replenished by weight. Sampling was accomplished at days 0, 2, 4, 8, 17, 31, 48 and 80 for spiked soils (non-aged and aged) and at days 0, 7, 14, 29, 60 and 88 for the industrial soil.

2.3 Extraction of PAH

For soil samples from the biodegradation experiment, extraction of PAH was carried out using a Soxhlet apparatus (GFL; Burgwedel, Germany) and dichloromethane (p.a., J. T. Baker; Deventer, Holland) as solvent (70 mL). Soil samples (5 g DM) were mixed thoroughly with the same amount of anhydrous Na_2SO_4 (p.a.; Fluka; Buchs, Switzerland) prior to extraction. Soxhlet extraction was performed over 20 hours.

2.4 Analysis of PAH

PAH were analyzed using HPLC (1050, Hewlett Packard; Waldbronn, Germany) equipped with a fluorescence detector (1046 A, Hewlett Packard; Waldbronn, Germany) and a diode array detector (1050, Hewlett Packard; Waldbronn, Germany) for non-fluorescent compounds. Chromato-

graphic separation of PAH was achieved using a reversed phase column (201TP54, 250 mm, 4.6 mm i.d., 5 μ m, VYDAC; Hesperia, CA, USA). A gradient starting with a 60:40 (v:v) ratio of acetonitrile:water with increasing acetonitrile to 100 % within 20 minutes was applied. Chromatograms were analyzed using Chemstation A.04.02 (Hewlett Packard; Waldbronn, Germany). All solvents (ultra gradient HPLC grade) were purchased from J. T. Baker; Deventer, Holland. PAH-standards were purchased from SUPELCO, Deisenhofen, Germany.

2.5 Sequential Supercritical Fluid Extraction (SSFE)

A stepwise extraction method using supercritical carbon dioxide (SFC TP, AGA; Vienna, Austria) as extraction fluid was developed to investigate the time dependent release of PAH from contaminated soil. Prior to extraction, soil samples were air dried for 16 hours at 20° C. Three to four grams of dried soil were introduced into an extraction thimble (7 mL) with a glass fiber filter (0.7 μ m, Osmonics; Minnetonka, MN, USA) on the bottom. To keep the void volume of the thimble constant, sea sand (p.a., acid purified, 40–200 mesh, Fluka; Buchs, Switzerland) was added to a total amount of 6 grams. Finally, the sand was covered with a second glass fiber filter to prevent clogging of tubings from fine particles.

Eight subsequent extraction steps comprising moderate extraction conditions (Table 4) were applied to contaminated soils using the SFE System HP 7680T (Hewlett Packard; Wilmington, DE, USA). A final extraction step using harsh extraction conditions with addition of 3 % methanol (J. T. Baker; Deventer, Holland) as a modifier was applied in order to remove residual contaminants from the matrix. Extracted PAH were trapped on an octadecylsilane (ODS, Hewlett Packard; Wilmington, DE, USA) and after each extraction step, the respective fractions were rinsed from the trap using a 1:1 (v:v) mixture of acetonitrile and tetrahydrofuran (ultra gradient HPLC grade, J. T. Baker; Deventer, Holland) and collected in individual vials. For the sequential extraction method four parallels were carried out and a coefficient of variance of 3 % was obtained for fluoranthene. Recoveries were expressed as the percentage of the respective PAH extracted under mild conditions (extraction steps 1 to 8) related to total amount of pollutant extracted under mild and harsh conditions (extraction steps 1 to 9).

Table 4: Supercritical fluid extraction conditions
Tabelle 4: Einsatzbedingungen der Superkritischen Fluid Extraktion

Method	initial steps	final step
No. of steps	8	1
Static extraction (min/step)	1	1
Dynamic extraction (min/step)	0.7	25
Density of CO ₂ (g/mL)	0.45	0.5
Temperature (°C)	100	130
Flow in thimble (mL/min)	3.2	3.8
Methanol added (%)	0	3

3. Results and discussion

When assuming that sequestration involves abiotic processes like diffusion and sorption, the bioavailable fraction of the contaminant should be predictable by the application of mild extractants (KELSEY et al., 1997). Moreover, sequential extraction procedures using supercritical fluids were applied to soil for the characterization of the sorption/desorption behavior of PCB (BJÖRKLUND et al., 1999; HAWTHORNE et al., 1999).

In this study two spiked model soils, both non-aged and aged as well as an industrial soil from a former manufactured gas plant site were selected to investigate the influence of the soil composition (in particular, the soil organic matter content) and the residence time of PAH in soil on the biodegradation performance. Moreover, a sequential extraction method was developed to evaluate the time dependent release of PAH from these soils, thus delivering information on the extractability of these contaminants sequestered in soil. Biodegradation data were compared to sequential extraction results. The aim of the study was to develop a fast, reliable, and cost efficient method for the prediction of the bioavailability of PAH in soil. As a model PAH, fluoranthene was chosen as it was the most abundant high molecular weight PAH in both, the anthracene oil as well as the industrial soil. Not only the abundance of this pollutant at PAH contaminated sites, but also its ability to cause DNA damaging effects when applying the Mutatox® test (JOHNSON, 1998) resulted in the selection of this contaminant.

3.1 Biodegradation results

When comparing biodegradation in spiked non-aged soils, a lower residual fluoranthene concentration was achieved in

soil 2 containing a higher amount of organic matter. Figure 1 indicates a 50 % reduction of fluoranthene in soil 1 (organic matter of 2.1 %) whereas an increased degradation of the contaminant (reduction of 78 %) in soil 2 (organic matter of 8 %) was observed. This indicates that no significant sequestration of PAH by SOM occurred due to immediate biodegradation right after spiking with anthracene oil. Thus, the availability of fluoranthene to degrading microorganisms was assumed to be roughly the same for both non-aged spiked model soils. The even enhanced biodegradation in soil 2 may be explained by the fact that the higher organic matter content provides optimum conditions for microbial growth. However, for aged soil 2 the biodegradation (80 days) of fluoranthene was dramatically reduced to 3 % (135 days of aging) compared to 78 % when non-aged. For aged soil 1 (low organic matter content) no effect of aging on the biodegradation could be observed (Figure 2) which supports the assumption that SOM significantly contributes to sequestration processes of hydrophobic pollutants in soil.

For the industrially contaminated soil (soil A), the microbial reduction of the fluoranthene concentration (23 %) was rather low compared to spiked soils. Results indicate a decreased bioavailability of the respective contaminant for

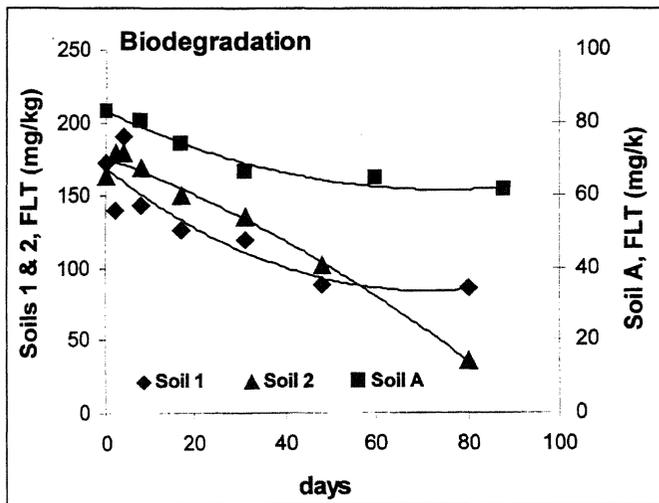


Figure 1: Biodegradation of fluoranthene (FLT) in non-aged spiked soils (soil 1, soil 2) and industrial soil (soil A); highest reduction of the fluoranthene concentration by biodegradation means was achieved for non-aged soil 2 containing 8 % of organic matter

Abbildung 1: Mikrobieller Abbau von Fluoranthen (FLT) in nicht gealterten kontaminierten Modellböden (soil 1, soil 2) sowie in Industrieböden (soil A); höchste Fluoranthen-Abbaureate wurde für nicht gealterten Boden 2 (soil 2) erzielt, welcher 8 % an organischer Substanz enthält

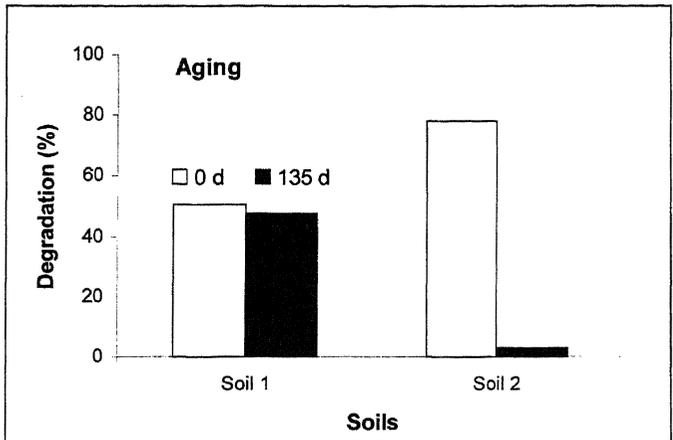


Figure 2: Biodegradation of fluoranthene (FLT) in spiked soils prior to (0 d) and after aging (135 d); aging reduced the percentage degraded from 78 to 3 % for soil 2 whereas no distinct change could be noticed for soil 1

Abbildung 2: Mikrobieller Abbau von Fluoranthen in kontaminierten Modellböden bevor (0 d) und nach Alterung (135 d); für Boden 2 (soil 2) wurde durch Alterung der Abbaugrad von 78 % auf 3 % reduziert. Kein Unterschied konnte für Boden 1 (soil 1) beobachtet werden

soils where aging has occurred for several decades (Figure 1). Only for aged soil 2, less fluoranthene was degraded when compared to the industrial soil. This might be due to the high organic matter content of the model soil which favors sequestration even at comparatively short aging periods (NAM et al., 1998).

3.2 Sequential Supercritical Fluid Extraction (SSFE)

Subsequent extraction steps were applied to contaminated soil samples in order to obtain information on the retention/release behavior of PAH in different types of soil prior to and after aging. Figure 3 shows the stepwise extraction of fluoranthene from the two spiked model soils aged for 135 days as well as from the industrial soil. For the aged soil 2, a reduced recovery of fluoranthene (9 % after eight steps) compared to soil 1 can be observed indicating stronger retention of the contaminant by the soil higher in organic matter soil. These findings correspond well to the results discussed in the previous paragraph, where degradation of fluoranthene was also reduced by the high organic matter soil after aging. For the industrial soil (soil A) the extractability was even lower than for aged soil 2, which does not exactly match the results obtained from the biodegradation experiment where soil 2 showed the lowest

degradability. However, in general, a high organic matter content and a long residence time of pollutants in soil will reduce the availability of the pollutants (NAM et al., 1998). No conspicuous difference in extraction behavior could be

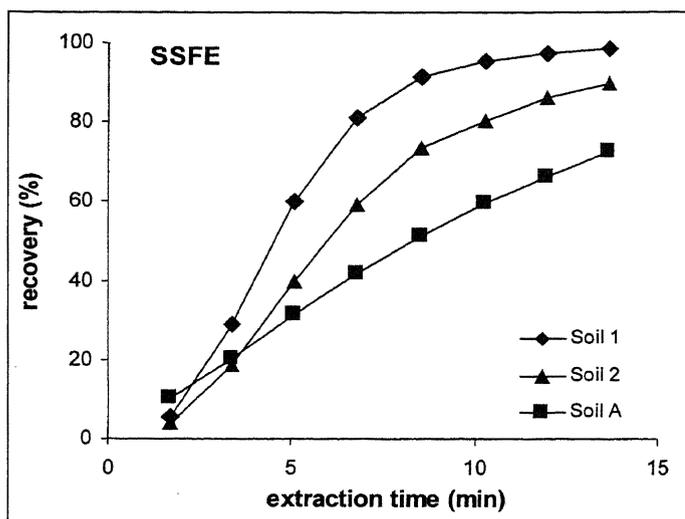


Figure 3: SSFE of fluoranthene (FLT) from aged spiked model soils (soil 1, soil 2) and industrial soil (soil A); reduced recovery of fluoranthene for soils with increased SOM content and extended aging period, respectively

Abbildung 3: SSFE von Fluoranthen (FLT) von kontaminierten Modellböden (soil 1, soil 2) sowie Industrieboden (soil A); reduzierte Wiederfindung von Fluoranthen wurde für Böden mit höherem Gehalt an organischer Substanz bzw. bei sehr langer Alterung beobachtet

seen for non-aged spiked model soils and soil 1 (low organic matter content) after aging. However, for soil 2, aging caused a reduction of the total fluoranthene recovery of 11 % after the last step (step 8) of the sequential extraction (moderate conditions).

Figure 4 deals with the stepwise extraction of 11 of the 16 US-EPA PAH from the industrial soil (soil A). With increasing molecular weight of PAH, the extraction efficiency is diminished in terms of a reduction of the recovery within the eight extraction steps applied. These data are in line with the concept that PAH with increasing molecular weight (increasing hydrophobicity) will be sorbed more effectively in/to soil organic matter.

When comparing biodegradation performance to extraction efficiency, the same trend was observed. Figure 5 represents the results achieved for the most abundant PAH in soil A that showed biodegradation. For compounds higher in ring number, no distinct biodegradation could be noticed within the course of the experiment.

Although microbial decay was very low for the industrial soil and the recovery after eight extraction steps applied (total extraction time of 13.6 min) was more than twice as high as the amount degraded, recovery and degradation exhibit good correlation with a reduced release and accessibility of PAH with increasing molecular weight. Besides

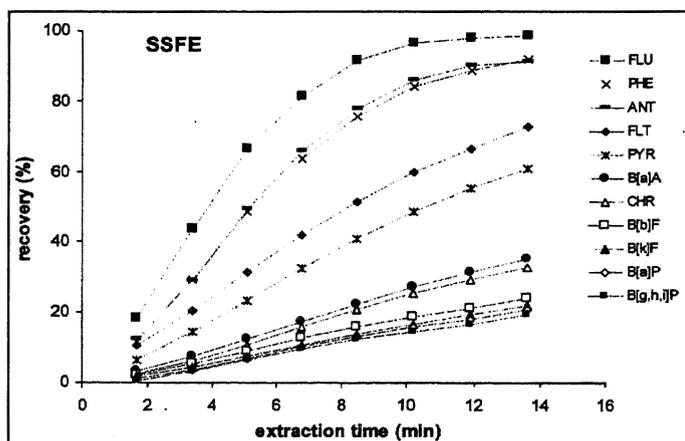


Figure 4: Subsequent release of PAH from industrial soil (soil A) by the application of SSFE; decreasing recovery for compounds with increasing molecular weight; full names of PAH abbreviations are given in Table 3

Abbildung 4: Zeitlicher Verlauf der Freisetzung von PAH aus Industrieboden (soil A) erzielt durch den Einsatz von SSFE; abnehmende Wiederfindung für Komponenten mit steigendem Molekulargewicht. Vollständige Namen der PAH sind in Tabelle 3 angegeben

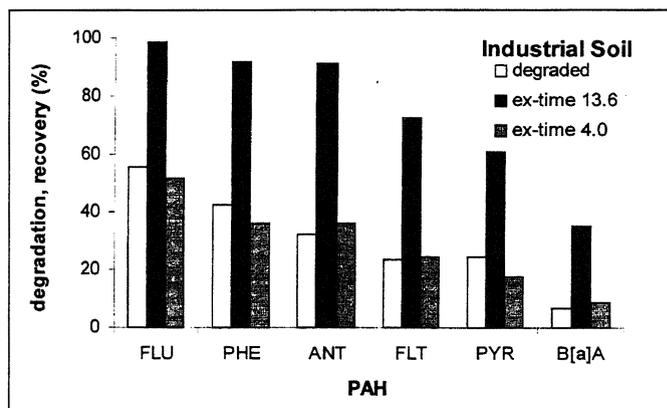


Figure 5: Comparison of biodegradation (degraded) and SSFE recovery for industrial soil; ex-time 13.6 reflects the amount of PAH recovered after a total extraction time (moderate conditions) of 13.6 min, ex-time 4.0 gives calculated data for an extraction time of four minutes; full names of PAH abbreviations are given in Table 3

Abbildung 5: Vergleich von biologischem Abbau (degraded) und SSFE-Wiederfindung für Industrieboden; 'ex-time 13.6' gibt die Menge an PAH an, die nach der Gesamtextraktionszeit von 13.6 min (milde Bedingungen) gefunden wurde, 'ex-time 4.0' gibt kalkulierte Werte für eine Extraktionszeit von 4 min an; vollständige Namen der PAH sind in Tabelle 3 angegeben

extraction recovery of PAH after 13.6 min (eight steps) and their reduction due to biodegradation, Figure 5 shows the amount of PAH extracted after four minutes (interpolated data). The results indicate that after an extraction time of four minutes, an equivalent amount of contaminants was extracted when compared to the quantity degraded.

4. Conclusions

From the results achieved for biodegradation experiments on spiked and industrially PAH contaminated soils, two main conclusions may be drawn. The phenomenon of aging caused by an increased residence time of pollutants in soil reduces the bioavailability in terms of the percentage biodegraded. Moreover, the content of organic matter affects the degradation of PAH in soil inversely. A high organic matter content shows low degradation yields, which could be seen for aged spiked soil samples only (Figure 2). However, for non-aged samples, biodegradation was more pronounced in the soil containing high organic matter (8 % SOM). This is most probably due to an increased microbial activity in soils rich in humic matter (Figure 1).

When applying SSFE, the content of organic matter influences the release of PAH from soil as well. Lower extraction yields expressed by the cumulative percentage of PAH extracted within the application of eight subsequent extraction steps under moderate extraction conditions were achieved for the spiked soil with high organic matter (Figure 3). This was true for aged spiked soils but could not be seen for non-aged samples. Moreover, by means of aging, a recovery reduction of 11 % was achieved for the high organic matter soil (8 % SOM) but could not be observed for the other model soil (2.1 % SOM). In addition to the soil composition, the chemical structure of the pollutants influences the retention/release behavior of PAH from industrial soil (Figure 4). Contaminants with high molecular weight exhibited reduced recoveries when applying SSFE, which might be explained by their chemical properties (in general decreasing polarity and ascending K_{OW} -coefficients).

Bioavailability of PAH as the predominant requirement for a successful bioremediation was estimated by relating SSFE recoveries to microbial degradation results for industrial soil (Figure 5). Although the extent of microbiological break-down was not as pronounced as the amount extracted, a comparable pattern of biodegradation success and extraction yield (recovery) could be observed. The percentage degraded was reflected more accurately by the amount

extracted after a distinct time of four minutes, indicating that further development of the SSFE by relating supercritical fluid conditions to desorption and transfer processes may result in an extraction procedure for a precise assessment of the bioavailable fraction.

Acknowledgments

This work was supported by the Framework Programme 4 of the European Union for research and technology development (ENV4-CT97-0602) and the 'Research Programme for Contaminated Sites in Austria' of the Österreichische Kommunalkredit (GZ 9820015).

References

- BJÖRKLUND, E., S. BØWADT, L. MATHIASSEN and S. B. HAWTHORNE (1999): Determining PCB Sorption/Desorption Behavior on Sediments Using Selective Supercritical Fluid Extraction. 1. Desorption from Historically Contaminated Samples. *Environ. Sci. Technol.* 33, 2193–2203.
- BONTEN, L. T. C., J. T. C. GROTENHUIS and W. H. RULKENS (1999): Enhanced Biodegradation of PAH Contaminants by Thermal Pretreatment. In: A. LEESON and B. C. ALLEMAN (Eds.): *Bioremediation Technologies for Polycyclic Aromatic Hydrocarbon Compounds*. Batelle Press, Columbus, Ohio, 265–270.
- BOUCHEZ, M., D. BLANCHET and J-P. VANDECASTEELE (1995): Degradation of Polycyclic Aromatic Hydrocarbons by Pure Strains and by Defined Strain Associations: Inhibition Phenomena and Cometabolism. *Appl. Microbiol. Biotechnol.* 43, 156–164.
- CAVALIERI, E. L. and E. G. ROGAN (1992): The Approach to Understanding Aromatic Hydrocarbon Carcinogenesis. The Central Role of Radical Cations in Metabolic Activation. *Pharmac. Ther.* 55, 183–199.
- CHUNG, N. and M. ALEXANDER (1998): Differences in Sequestration and Bioavailability of Organic Compounds Aged in Dissimilar Soils. *Environ. Sci. Technol.* 32, 855–860.
- CHUNG, N. and M. ALEXANDER (1999): Effect of Concentration on Sequestration and Bioavailability of Two polycyclic Aromatic Hydrocarbons. *Environ. Sci. Technol.* 33, 3605–3608.
- CONOLLY, M., F. HOWE and M. MAZUR (1999): Full Scale

- Bioremediation of PAH. In: A. LEESON and B. C. ALLEMAN (Eds.): *Bioremediation Technologies for Polycyclic Aromatic Hydrocarbon Compounds*. Batelle Press, Columbus, Ohio, 43–49.
- CYPERS, M. P., J. T. C. GROTEHUIS and W. H. RULKENS (1999): Prediction of PAH Bioavailability in Soils and Sediments by Persulfate Oxidation. In: A. LEESON and B. C. ALLEMAN (Eds.): *Bioremediation Technologies for Polycyclic Aromatic Hydrocarbon Compounds*. Batelle Press, Columbus, Ohio, 241–246.
- ESCHENBACH, A., R. WIENBERG and B. MAHRO (1998): Fate and Stability of Nonextractable Residues of [¹⁴C]PAH in Contaminated Soils under Environmental Stress Conditions. *Environ. Sci. Technol.* 32, 2585–2590.
- GILLETTE, J. S., R. G. LUTHY, S. J. CLEMETT and R. N. ZARE (1999): Direct Observations of Polycyclic Aromatic Hydrocarbons on Geosorbents at the Subparticle Scale. *Environ. Sci. Technol.* 33, 1185–1192.
- HATZINGER, P. B. and M. ALEXANDER (1997): Biodegradation of Organic Compounds Sequestered in Organic Solids or in Nanopores within Silica Particles. *Environmental Toxicology and Chemistry* 16(11), 2215–2221.
- HAWTHORNE, S. B., E. BJÖRKLUND, S. BOWADT and L. MATHIASSEN (1999): Determining PCB Sorption/Desorption Behavior on Sediments Using Selective Supercritical Fluid Extraction. 3. Sorption from Water. *Environ. Sci. Technol.* 33, 3152–3159.
- HUESEMANN, M. H. (1997): Incomplete Hydrocarbon Biodegradation in Contaminated Soils: Limitations in Bioavailability or Inherent Recalcitrance. *Bioremediation Journal* 1(1), 27–39.
- JOHNSON, B. T. (1998): Microtox Toxicity Test System – New Developments and Applications. In: P. G. WELLS, K. LEE and C. BLAISE (Eds.): *Microscale Testing in Aquatic Toxicology*. CRC Press LLC, Boca Raton, Florida, 201–218.
- KELSEY, J. W., B. D. KOTTLER and M. ALEXANDER (1997): Selective Chemical Extractants to Predict Bioavailability of Soil-Aged Organic Chemicals. *Environ. Sci. Technol.* 31, 214–217.
- LI, X-F., X-C. LE, C. D. SIMPSON, W. R. CULLEN and K. J. REIMER (1996): Bacterial Transformation of Pyrene in a Marine Environment. *Environ. Sci. Technol.* 30, 1115–1119.
- LOIBNER, A. P., M. GARTNER, M. SCHLEGL, I. HAUZENBERGER and R. BRAUN (1997): PAHs: Rapid Estimation of their Bioavailability in Soil. In: B. C. ALLEMAN and A. LEESON (Eds.): *In Situ and On-Site Bioremediation*, Vol. 5, Batelle Press, Columbus, Ohio, 617–622.
- LOIBNER, A. P., O. SZOLAR, M. SCHLEGL and R. BRAUN (1998): Bioavailability of PAHs in Soil and Ecotoxicological Considerations. *Contaminated Soil '98*, Vol. 2, Thomas Telford Publishing, London, 797–799.
- LUTHY, R.G., G. R. AIKEN, M. L. BRUSSEAU, S. D. CUNNINGHAM, P. M. GSCHWEND, J. J. PIGNATELLO, M. REINHARD, S. J. TRAINA, W. J. WEBER JR. and J. C. WESTALL (1997): Sequestration of Hydrophobic Organic Contaminants by Geosorbents. *Environ. Sci. Technol.* 31, 3341–3347.
- NAM, K. and M. ALEXANDER (1998): Role of Nanoporosity and Hydrophobicity in Sequestration and Bioavailability: Tests with Model Solids. *Environ. Sci. Technol.* 32, 71–74.
- NAM, K., N. CHUNG and M. ALEXANDER (1998): Relationship between Organic Matter Content of Soil and the Sequestration of Phenanthrene. *Environ. Sci. Technol.* 32, 3785–3788.
- PIGNATELLO, J. J. and B. XING (1996): Mechanisms of Slow Sorption of Organic Chemicals to Natural Particles. *Environ. Sci. Technol.* 30, 1–11.
- SCHNEIDER, J., R. GROSSER, K. JAYASIMHULU, W. XU and D. WARSHAWSKY (1996): Degradation of Benz[a]anthracene, and Benz[a]pyrene by *Mycobacterium* sp. Strain RJGII-135, Isolated from a Former Coal Gasification Site. *Applied and Environmental Microbiology* 62(1), 13–19.
- SHAW, G. R. and D. W. CONNELL (1994): Prediction and Monitoring of the Carcinogenicity of Polycyclic Aromatic Compounds (PACs). *Reviews of Environmental Contamination and Toxicology* 135, 1–62.
- SMITH, M. R. (1990): The Biodegradation of Aromatic Hydrocarbons by Bacteria. *Biodegradation* 1, 191–206.
- TANG, J., B. K. ROBERTSON and M. ALEXANDER (1999): Chemical-Extraction-Methods to Estimate Bioavailability of DDT, DDE, and DDD in Soil. *Environ. Sci. Technol.* 33, 4346–4351.
- VOLKERING, F., J. J. QUIST, A. F. M. VAN VELSEN, P. H. G. THOMASSEN and M. OLIJVE (1998): A Rapid Method for Predicting the Residual Concentration after Biological Treatment of Oil-Polluted Soil. In: *Contaminated Soil '98*, Vol. 1, Thomas Telford Publishing, London, 251–259.
- WARSHAWSKY, D., T. CODY, M. RADIKE, R. REILMAN, B. SCHUHMAN, K. LADOW and J. SCHNEIDER (1995): Bio-transformation of Benzo[a]pyrene and Other Polycyclic

- Aromatic Hydrocarbons and Heterocyclic Analogs by Several Green Algae and Other Algal Species under Gold and White Light. *Chemico-Biological Interactions* 97, 131–148.
- WEISSENFELS, W. D., H.-J. KLEWER and J. LANGHOFF (1992): Adsorption of Polycyclic Aromatic Hydrocarbons (PAHs) by Soil Particles: Influence on Biodegradability and Biototoxicity. *Applied Microbiology and Biotechnology* 36, 689–696.
- WHITE, J. C., A. QUINONES-RIVERA and M. ALEXANDER (1998a): Effect of Wetting and Drying on the Bioavailability of Organic Compounds Sequestered in Soil. *Environmental Toxicology and Chemistry* 17(12), 2378–2382.
- WHITE, J. C., M. ALEXANDER and J. J. PIGNATELLO (1998b): Enhancing the Bioavailability of Organic Compounds Sequestered in Soil and Aquifer Solids. *Environmental Toxicology and Chemistry* 18(2), 182–187.
- WINNINGHAM, J., R. BRITTO, M. PATEL and F. MCINTURFF (1999): A Landfarming Field Study of Creosote-Contaminated Soil. In: A. LEESON and B. C. ALLEMAN (Eds.): *Bioremediation Technologies for Polycyclic Aromatic Hydrocarbon Compounds*. Batelle Press, Columbus, Ohio, 37–42.
- XING, B. and J. J. PIGNATELLO (1997): Dual-Mode Sorption of Low Polarity Compounds in Glassy Poly(Vinyl Chloride) and Soil Organic Matter. *Environ. Sci. Technol.* 31, 792–799.
- XING, B. and J. J. PIGNATELLO (1998): Competitive Sorption between 1,3-Dichlorobenzene or 2,4-Dichlorophenol and Natural Aromatic Acids in Soil Organic Matter. *Environ. Sci. Technol.* 32, 614–619.
- YE, D., M. A. SIDDIQI, A. E. MACCUBBIN, S. KUMAR and H. C. SIKKA (1996): Degradation of Aromatic Hydrocarbons by *Sphingomonas paucimobilis*. *Environ. Sci. Technol.* 30, 136–142.

Address of authors

Dr. Andreas P. Loibner, Dipl.-Ing. Michaela Holzer, Dr. Michael Gartner, Dipl.-Ing. Oliver H.J. Szolar and Ao. Univ.-Prof. Dr. Rudolf Braun, IFA-Tulln, Department of Environmental Biotechnology, Konrad Lorenz Str. 20, A-3430 Tulln, Austria; e-mail: loibner@ifa-tulln.ac.at

Eingelangt am 18. Juli 2000

Angenommen am 30. Oktober 2000