Anaerobic biodegradation of aromatic hydrocarbons in groundwater

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Short Summary

The crude oil constituents benzene, toluene, ethylbenzene, and the three xylene isomers (BTEX) are the dominating groundwater contaminants originating from surface spill accidents by oil production facilities and with gasoline and jet fuel. Thereby BTEX posing a threat to the world´s scarce drinking water resources due to their water solubility and toxicity. An active remediation cleanup involving a BTEX event proves not only to be very expensive but almost impossible when it comes to the complete removal of contaminants from the subsurface. A favoured and common practice is combining an active remediation process focussing on the source of contamination coupled together with the monitoring of the residual contamination in the subsurface (monitored natural attenuation; MNA). MNA include all naturally occuring biological, chemical and physical processes in the subsurface.

The general goal of this work was to improve the knowledge of biodegradation of aromatic hydrocarbons under anaerobic conditions in groundwater. For this groundwater and soil at the former military underground storage tank (UST) site Schäferhof–Süd near Nienburg/Weser (Niedersachsen, Germany) were sampled and analysed. The investigations were done in collaboration of the Umweltbundesamt, the universitys of Frankfurt and Bremen and the alphacon GmbH Ganderkesee.

To investigate the extent of groundwater contamination, the terminal electron acceptor processes (TEAPs) and the metabolites of BTEX degradation in groundwater, six observation wells were sampled at regular intervals between January 2002 and September 2004. The wells were positioned in order to cover the upstream, the source area and the downstream of the presumed contamination source. Additionally, vertical sediment profiles were sampled and investigated with respect to spreading and concentration of BTEX in the subsurface.

A large residual contamination involving BTEX is present in soil and groundwater at the studied locality. Maximum BTEX concentration values of 17 mg/kg were recorded in analysing sediment in the unsaturated zone. In the capillary fringe, values of 450 mg/kg were recorded (October 2004) and in the saturated zone maximum values of 6.7 mg/kg BTEX were detected. The groundwater samples indicate increasing BTEX concentrations in the groundwater flow direction (from 532 µg/l up to 3300 µg/l (mean values)).

Biodegradation of aromatic hydrocarbons under anaerobic conditions in the sub-

surface at contaminated sites is characterised by generation of metabolites. From the monoaromatic hydrocarbons BTEX metabolites such as benzoic acid (BA) and the methylated homologs and C_1-and C_2-benzyl-succinic acids (BSA) are generated as intermediates. A solid-phase extraction method based on octadecyl-bonded silica sorbent has been developed to concentrate such metabolite compounds from water samples followed by derivatization and gas chromatography/mass spectrometry (GC/MS) of the extracts. The recovery rate range between 75 and 97%. The method detection limit was 0.8 µg/l.

Organic acids were identified as metabolic by-products of biodegradation. Benzoic acid, C_1-, C_2- and C_3-benzoic acid were determined in all contaminated wells with considerable concentrations. Furthermore, the depletion of the dominant terminal electron acceptors (TEAs) oxygen, nitrate, and sulphate and the production of dissolved ferrous iron and methane in groundwater indicate biological mediated processes in the plume evidently proving the occurrence of NA. A large overlap of different redox zones at the studied part of the plume has been observed.

A important finding in this study is the strong influence of groundwater level fluctuations on the BTEX concentration in groundwater. A very dry summer in 2003 was recorded during the monitoring period, resulting on site in a drop of the groundwater level to 1.7 m and a concomitant increase of BTEX concentrations from 240 µg/l to 1300 µg/l. The groundwater level fluctuations, natural degradation and retention processes essentially influence BTEX concentrations in the groundwater. Groundwater level fluctuations have by far a stronger influence than the influence of biological degradation. Increasing BTEX concentrations are hence not a consequence of limited biological degradation.

Another part of the study was to observe the isotopic fractionation of the electron acceptor Fe(III), due to biologically mediated reduction of Fe(III) to the water-soluble Fe(II) at the site and first field data are presented. Both groundwater and sediment samples were analysed with respect to their Fe isotopic compositions using high mass resolution Multi Collector-Inductively Coupled Plasma-Mass Spectrometry (MC-ICP-MS).

The δ^{56}Fe-values of groundwater samples taken from observation wells located downstream of the source area were isotopically lighter than δ^{56}Fe-values obtained from groundwater in the uncontaminated well. The Fe isotopic composition of most

parts of the sediment profile was similar to the Fe isotopic composition of uncontaminated groundwater. Thus, a significant iron isotope fractionation can be observed between sediment and groundwater downstream of the BTEX contamination.

Kurzfassung (Short Summary)

In der vorliegenden Arbeit wurden geochemische Prozesse zum biologischen Abbau von aromatischen Kohlenwasserstoffen im Grundwasser am Standort Schäferhof–Süd (Nienburg/Weser in Niedersachsen) untersucht. Hierzu wurden auf dem ehemals militärisch genutzten Gelände Grundwasser- und Bodenproben entnommen, umfangreich analysiert und ausgewertet.

Die aromatischen Kohlenwasserstoffe Benzol, Toluol, Ethylbenzol und die Isomere des Xylols (BTEX) sowie Mineralölkohlenwasserstoffe (MKW) weisen ein hohes toxisches Potential auf. Benzol ist hinreichend als Karzinogen bekannt. Durch Leakagen und dem unsachgemäßem Umgang mit Mineralölen und Mineralölprodukten kommt es häufig zu Kontaminationen des Bodens und Grundwassers, wodurch oft Trinkwasserressourcen bedroht werden. Aus diesem Zusammenhang heraus erfordern Mineralölschäden eine genaue Untersuchung bezüglich des Kontaminationsherdes und der Ausbreitung der Schadstoffe im Untergrund. Im Anschluss daran ist der Einsatz von aktiven und überwachenden Sanierungsmassnahmen erforderlich. Hier hat sich in der Vergangenheit der Ansatz des 'Kontrollierten Abbaus und Rückhalt von Schadstoffen' (Monitored Natural Attenuation; MNA) bewährt, welcher die natürlich ablaufenden biologischen, chemischen und physikalischen Prozesse im Untergrund berücksichtigt.

Um die Kontamination durch BTEX und die biologischen Abbauprozesse im Grundwasser am ausgewähltem Standort zu untersuchen, wurden auf dem Areal insgesamt sechs Grundwassermessstellen über einen Zeitraum von drei Jahren (2002-2004) regelmäßig beprobt und analysiert. Bei den chemischen Analysen standen neben den Schadstoffen die chemischen Komponenten Sauerstoff, Nitrat, Eisen(II), Sulfat und Methan, sowie die Abbauprodukte von BTEX im Vordergrund. Im Bereich des vermuteten Haupteintragsherdes der Kontamination wurden mittlere BTEX-Konzentrationen von 532 µg/l im Grundwasser gemessen. Diese stiegen in Grundwasserfließrichtung bis auf 3300 µg/l (Mittelwert) im oberen Bereich des Grundwasserleiters an, was auf zusätzliche, stromabwärts gelegene Eintragsquellen von BTEX hinwies.

Der direkte Nachweis von biologischen Abbauprodukten (organische Säuren), welche durch den mikrobiellen Abbau von BTEX und von polyzyklischen aroma-

tischen Kohlenwasserstoffen entstehen, ist eine gute und anerkannte Methode im Bereich MNA zum Nachweis mikrobieller Abbauprozesse im Grundwasser. Die Analytik dieser organischen Säuren aus dem Grundwasser erfolgt durch Gaschromatographie gekoppelt mit Massenspektrometrie (GC/MS). Im Rahmen der Dissertation wurde ein Verfahren für die Festphasenextraktion entwickelt, welches den Arbeitsablauf der Extraktion von Metaboliten aus dem Grundwasser erheblich vermindert. Die Wiederfindungsraten der Methode liegen zwischen 75-97% und die Nachweisgrenzen bei 0,8 µg/l.

Im Anstrom und in den tief verfilterten Bereichen des Grundwasserleiters wurden nur geringe Konzentrationen von Metaboliten nachgewiesen, welche natürlichen Hintergrundwerten entsprechen. In den flach verfilterten Abschnitten des Grundwasserleiters traten jedoch im kontaminierten Bereich erhöhte Konzentrationen von Benzoesäure und C_1-C_3-Benzoesäuren auf. Diese korrelierten mit den erhöhten Substratgehalten im Grundwasser. Weiterhin belegte die Zehrung der Elektronenakzeptoren O_2, NO_3^-, Fe^{3+}, SO_4^{2-} und der Ablauf von Methanogenese im kontaminierten Grundwasser den mikrobiellen Schadstoffabbau unter anaeroben Milieubedingungen. Die Ergebnisse zeigen, dass steigende BTEX-Konzentrationen im Grundwasser am Standort nicht die Folge eines eingeschränkten biologischen Abbaus sind.

Als eine weitere wichtige Erkenntnis konnte eine intensive Abhängigkeit der BTEX-Konzentrationen im Grundwasser von der Änderung des Grundwasserstandes am Standort festgestellt werden. Im Bereich des Haupteintragsherdes von BTEX wurde eine negative Korrelation der Schadstoffkonzentrationen mit der Höhe des Grundwasserstandes beobachtet. Bedingt durch das sehr trockene Sommerhalbjahr 2003, kam es zu einer Absenkung des Grundwasserspiegels um 1,7 m im Vergleich zum vorhergehenden Winterhalbjahr, was zur Folge hatte, dass die BTEX-Konzentrationen am Ort des Eintrages der Kontamination von 240 µg/l auf 1300 µg/l im Grundwasser anstiegen. Die BTEX-Konzentrationen im Grundwasser werden von den natürlichen Abbau- und Rückhalteprozessen im Untergrund beeinflusst, jedoch war am Standort der Einfluss von Grundwasserschwankungen deutlich stärker als die NA-Prozesse.

Um die Erkenntnisse der im Boden ablaufenden Abbauprozesse zu erweitern, wurde der Zusammenhang der Fraktionierung von Eisenisotopen während der Re-

duktion von gebundenem Eisen(III) im Sediment zu wasserlöslichem Eisen(II) im Grundwasser untersucht. Es wurde festgestellt, dass unkontaminierte Grundwasserproben und kontaminierte Bodenproben ähnliche Isotopien aufweisen. Die δ^{56}Fe Werte der kontaminierten Grundwasserproben, welche stromabwärts des vermuteten BTEX-Haupteintragsherdes genommen wurden, sind isotopisch signifikant leichter als die kontaminierten Bodenproben. Die auseinandergehenden Isotopien der Grundwasser- und Bodenproben gaben Anhaltspunkte für die mikrobielle Aktivität im Grundwasser.

Zusammenfassung (Summary)

Kontaminationen in der Umwelt durch Mineralöle und Mineralölprodukte gehören heute überall in der Welt zu den größten Aufgaben und Problemen von Sanierungsvorhaben. Bedingt durch den jahrelangen unsachgemäßen Umgang und wiederholten Unfällen mit Schadstoffen, wie z.B. Benzin oder Dieselkraftstoffen sind Verunreinigungen von Böden, Grund- und Oberflächenwasser und der Athmosphäre weit verbreitet. Insbesondere auf ehemals militärisch genutzten Liegenschaften wurde der Untergrund durch den Militärbetrieb häufig erheblich mit Mineralölkohlenwasserstoffe (MKW) und den aromatischen Kohlenwasserstoffen Benzol, Toluol, Ethylbenzol und den Xylol-Isomeren (nachstehend als BTEX bezeichnet) verunreinigt.

Dabei stellen vorallem die in Wasser partiell löslichen Kohlenwasserstoffe BTEX eine toxische und kanzerogene Gefahr dar. Besonders betroffen ist meist das Grundwasser und somit auch die Trinkwasserressourcen, da die Stoffe eine starke Tendenz zur Anreicherung im Sediment zeigen und durch Auswaschungsprozesse mit dem Sickerwasser wiederum über einen langen Zeitraum ins Grundwasser eingetragen werden können. Dadurch entsteht eine Gefährdung für die Umwelt und den darin lebenden Organismen.

Eine aktive Dekontaminierung von Boden und Grundwasser bei Schadensfällen, welche mit dem Eintrag von Kohlenwasserstoffen in die Bodenzone verbunden sind, ist häufig sehr kostenintensiv. Eine vollständige Entfernung der Kontaminanten aus dem Untergrund ist nur selten möglich. Konventionelle Ansätze wie z.B. ”pump-and-treat” Methoden haben in der Vergangenheit nur bedingt zu einem Sanierungserfolg geführt. Daher wird an solchen Standorten immer häufiger eine aktive Sanierung der Schadenszentren mit einem Monitoring der Restkontamination in der ungesättigten und gesättigten Bodenzone verbunden.

Der Ansatz für dieses Monitoring basiert auf der Tatsache, dass unter entsprechenden Bedingungen im Boden und Grundwasser die Menge, Toxizität und/oder die Mobilität von Kontaminanten ohne aktive Maßnahmen reduziert werden kann. Dies ist möglich, wenn biologische, physikalische und chemische Prozesse aktiv sind. Der kontrollierte Ablauf dieser natürlichen Abbau- und Rückhalteprozesse wird unter dem Begriff Monitored Natural Attenuation (MNA) zusammengefasst. Im Rahmen von MNA wird der biologische Abbau von Kohlenwasserstoffen durch

Mikroorganismen zur Dekontaminierung des Bodens und des Grundwassers genutzt. Mikroorganismen verwerten organische Moleküle wie z.B. BTEX zur Deckung des Energiebedarfs und zum Aufbau der eigenen Biomasse. Der dabei als Elektronenakzeptor genutzte Sauerstoff ist aufgrund seiner geringen Löslichkeit in Wasser nur begrenzt verfügbar. Daher verläuft der Schadstoffabbau hauptsächlich unter anaeroben Bedingungen durch die Reduzierung von Nitrat, Eisen(III) und Sulfat sowie durch Methanogenese. Dabei wird der biologische Umsatz der Kontaminanten hauptsächlich durch die Verfügbarkeit dieser alternativen Elektronenakzeptoren gesteuert.

In den letzten Jahren ist MNA als Strategie zur Dekontamination verstärkt ins Blickfeld des Interesses gerückt. So entspricht es auch dem Bundes-Bodenschutzgesetz, wenn eine nachhaltige Wiederherstellung der Bodenfunktionen mit natürlichen Schadstoffminderungsprozessen (neben technischen Sanierungsmaßnahmen) als umwelt- & bodenschonende und kostengünstige Methode bewusst und kontrolliert eingesetzt werden kann (BBodSchG, 1998).

Die vorliegende Dissertation wurde auf der Grundlage des Projektes "Langzeituntersuchungen zu den Möglichkeiten und Grenzen der Nutzung natürlicher Selbstreinigungsprozesse für ausgewählte Schadstoffe am Beispiel kontaminierter militärischer Liegenschaften (FKZ 298 76 712 /02)" welches vom Umweltbundesamt (UBA) im Zeitraum von 2001 bis 2004 durchgeführt wurde, erstellt. In diesem Projekt arbeiteten das Umweltbundesamt, das Zentrum für Umweltforschung und Umwelttechnologie der Universität Bremen, die Johann Wolfgang Goethe-Universität und die alphacon GmbH aus Ganderkesee zusammen, wobei das Umweltbundesamt die Koordination und Betreuung des Projektes inne hatte, die Universität Bremen die ungesättigte Bodenzone (Hettwer, 2006) und die Universität Frankfurt die gesättigte Bodenzone bearbeitete. Die alphacon GmbH übernahm die Probennahme und den überwiegenden Teil der Analytik.

Auf dem früher militärisch genutzten Gelände des Tanklagers (TL) Schäferhof–Süd bei Nienburg/Weser (Niedersachsen) wurden im Bereich einer rückgebauten ehemaligen Dieselabfüllstation für Kraftstoffe mit Lagerschuppen Untersuchungen zum biologischen Abbau von aromatischen Kohlenwasserstoffen und die Änderung

der Schadstoffkonzentrationen im Grundwasser im Zeitraum zwischen Januar 2002 und September 2004 durchgeführt. Ziel dieser Arbeit war es, die Kenntnisse über die geochemischen Prozesse im kontaminierten Grundwasserleiter und die Wechselwirkungen zwischen Grundwasser und Boden durch den mikrobiologischen Abbau von Schadstoffen zu erweitern. Hierfür wurde die Analytik von Metaboliten im Grundwasser durch ein modifiziertes Extraktionsverfahren verbessert.

Das seit 1976 ungenutzte Tanklager wurde in den Jahren 1995-2001 umfangreich rückgebaut. Dabei wurden die unterirdischen Tankanlagen, Rohrleitungen und Gebäude entfernt und die Mineralölprodukte umfangreich entsorgt. Trotzdem weist das gesamte Gelände am Untersuchungsstandort eine hohe Restkontamination der Verbindungen BTEX und Mineralölkohlenwasserstoffe (MKW) in der ungesättigten Bodenzone auf. Auf einer Fläche von ca. 10 x 25 m, auf welcher sich bis 1976 eine Dieselabfüllstation befand, wurde ein Testfeld angelegt. Hier wurde ab einer Tiefe von 1,30 m stark kontaminierter Boden angetroffen. Die ermittelten MKW-Gehalte lagen zu Beginn der Bodenuntersuchungen (2001) zwischen 87 und 5450 mg/kg, die BTEX-Gehalte zwischen 1,5 und 109 mg/kg Trockensubstanz.

Geologisch befindet sich das Untersuchungsgebiet in der Talniederung der Mittelweser, welche in diesem Naturraum den Vorfluter bildet. Der Grundwasserleiter ist aus fein- bis grobsandigen und kiesigen quartären Sanden aufgebaut, in welchem nicht durchgehende Tonlinsen eingeschaltet sind. Die Sedimente weisen einen k_f-Wert von 10^{-4} auf und der Grundwasserflurabstand variiert zwischen 4-17 m.

Um die Kontamination durch BTEX und die biologischen Abbauprozesse im Grundwasser am Standort Schäferhof–Süd zu untersuchen, wurden auf dem Areal insgesamt sechs Grundwassermessstellen (GWM) in regelmässigen Abständen von drei Monaten beprobt und analysiert. Um sowohl das Grundwasser im Anstrombereich, am Haupteintragsherd (Testfeld) und Abstrom beproben zu können, wurden die Messstellen entlang einer Transekte über das Gelände verteilt. Die GWM MP1-MP5 wurden als Messstellengruppen ausgebaut, was eine tiefenorientierte Beprobung in zwei verschiedenen Tiefen (4-7 m u.GOK und 9-10 m u.GOK) des Grundwasserleiters zulässt. Die Messstelle B8 ist als durchgehend verfilterte GWM

ausgebaut (4-12 m u.GOK). Die Untersuchungen beinhalteten Abstichmessungen, Bestimmung der physiko-chemischen Parameter, Messung der hydrochemischen Hauptinhaltsstoffe und der Schadstoffgehalte sowie deren Abbauprodukte. Das untersuchte Schadstoffspektrum umfasste MKW und BTEX, wobei MKW in allen Messstellen während des Beobachtungszeitraumes nur in vernachlässigbaren Konzentrationen oder unterhalb der Bestimmungsgrenze vorlag. Daher konzentrieren sich die Auswertungen in dieser Arbeit auf die BTEX-Gehalte im Grundwasser.

Die GWM MP1 im Anstrom war über den gesamten Monitoringzeitraum schadstofffrei und diente daher in den vorliegenden Untersuchungen als Referenz für die anderen, mit BTEX kontaminierten GWM. In den oberen Schichten des Grundwasserleiters war in Grundwasserfließrichtung im gesamten Monitoringzeitraum ein Anstieg der BTEX-Konzentrationen zu beobachten. Im Bereich des Testfeldes wurden mittlere BTEX-Konzentrationen von 532 µg/l im Grundwasser gemessen. Diese stiegen im Abstrombereich im flach verfilterten Bereich auf 3300 µg/l (Mittelwert) an. In den tief verfilterten Messstellen wurden, mit Ausnahme der Messstelle MP5 im Abstrom, keine Schadstoffe nachgewiesen. Die BTEX-Konzentrationen im Grundwasser an den einzelnen Messstellen zeigten an, dass mit dem bestehenden Messstellenaufbau die Abstromfahne nur zu einem Teil erfasst wurde und weitere Eintragsquellen aus der ungesättigten Bodenzone vorliegen müssen, was den Konzentrationsanstieg in Fließrichtung erklärt. Daraus ergab sich für das Tanklager eine gesonderte Situation, welche die Bewertung der biologischen Abbauprozesse erschwerte und eine detaillierte Untersuchung erforderte.

Untersuchungen zur Belastung des Bodens wurden auf dem Testfeld an acht Bohrarealen durchgeführt. Mittels Rammkernsondierungen wurden Bodenproben mit einer Profiltiefe bis zu 8 m u.GOK genommen. In dieser Arbeit wurden zwei der Bodenprofile (B3 und B5), welche in unmittelbarer Nähe zur GWM MP2 (Testfeld) im September 2004 abgeteuft wurden, analysiert und ausgewertet.

Die BTEX-Analysen der Bodenproben ergaben in der ungesättigten Bodenzone Maximalwerte von 17 mg/kg (Bohrpunkt B3/3) und erreichten im Kapillarraum Gehalte von 120 mg/kg (Bohrpunkt B3/3), während in der gesättigten Bodenzone Maximalwerte von 6,7 mg/kg (Bohrpunkt B3/4) nachgewiesen wurden. Aus diesen

Analysen der Bodenproben ging für das Testfeld eine vertikal stark inhomogene Verteilung der BTEX-Gehalte hervor. In den Bohrungen war deutlich ein nahezu sprunghafter Konzentrationsanstieg im Kapillarraum zu erkennen, was auf die bedingte Löslichkeit von BTEX zurückzuführen war. Die Schadstoffe wurden mit dem Sickerwasser aus der ungesättigten Bodenzone ausgewaschen und in den Kapillarraum verlagert, von wo aus ein langsamer Eintrag ins Grundwasser erfolgte. Die höchsten Schadstoffkonzentrationen wurden in einer Profiltiefe von 5-6 m gemessen. Dieser Tiefenbereich entspricht dem Niedrigststand des Grundwassers im Beobachtungszeitraum. Zwischen 2002 und 2004 traten durch das sehr trockene Sommerhalbjahr 2003 starke Grundwasserschwankungen mit einer Amplitude von bis zu 1,7 m auf. Diese nahmen einen erheblichen Einfluss auf die Schadstoffgehalte im Grundwasser. In diesem Sommer stiegen die BTEX-Konzentrationen im Grundwasser im Bereich des Testfeldes, welches als Haupteintragsquelle für die BTEX-Kontamination vermutet wurde, von 240 µg/l (März 2003) auf 1300 µg/l (Sept. 2003) an. Die Ganglinien des Grundwasserstandes und der BTEX-Konzentrationen verlaufen an der Messstelle MP2 gegenläufig zueinander, das heißt, bei steigendem Grundwasserstand sinken die BTEX-Gehalte, während sie bei sinkendem Grundwasserstand wieder ansteigen. Dieser Konzentrationsanstieg von BTEX warf die Frage auf, ob im Grundwasser tatsächlich natürliche Abbauprozesse ablaufen und welchen Einfluss die Grundwasserschwankungen auf die BTEX-Konzentrationen haben.

Innerhalb der Untersuchungen wurde ermittelt, dass sich bei niedrigen Grundwasserständen die Grundwasseroberfläche im Bereich der höchsten Bodenkontamination befand. Dadurch wurden in trockenen Perioden die BTEX-Konzentrationen im Grundwasser erhöht, während es bei höheren Grundwasserständen durch Verdünnungsprozesse zu einer BTEX-Konzentrationsabnahme im Grundwasser kam. In niederschlagsreichen Perioden war die Wassersäule höher und damit der vom Grundwasser durchströmte Bereich des kontaminierten Untergrundes größer. Jedoch wurden durch die inhomogene vertikale Schadstoffverteilung in diesem Fall auch die geringer kontaminierten Schichten von Grundwasser durchströmt. Bei hohen Grundwasserständen wurden folglich die aus der Bodenkontamination in Lösung gegangenen Schadstoffe (BTEX) stärker verdünnt als dies bei niedrigen Grundwasserständen möglich war.

Der mikrobielle Schadstoffabbau findet zunächst unter Zehrung von Sauerstoff im aeroben Milieu statt. Ist dieser verbraucht, geht die Mineralisierung der Schadstoffe ins anaerobe Milieu über und findet unter Reduzierung von Nitrat, Eisen(III) und Sulfat, sowie durch Methanogenese statt. Somit können die redoxsensitiven Parameter O_2, NO_3^-, Fe^{2+}, SO_4^{2-} und CH_4 im Grundwasser als Indikatoren für biologische Abbauvorgänge im Grundwasser verwendet werden. Verringerte Konzentrationen bzw. ein Anstieg der einzelnen Parameter in kontaminierten Grundwasserproben im Vergleich zu unkontaminiertem Grundwasser, geben Auskunft über die biochemischen Prozesse im Untergrund.

Am TL Schäferhof-Süd zeigten die genannten Parameter charakteristische Zu- und Abnahmen an den kontaminierten Messstellen im Vergleich zur schadstofffreien Messstelle MP1. So war in allen kontaminierten Messstellen eine starke Sauerstoff- und Nitratzehrung zu beobachten. Auch die intensive Sulfatreduktion (von 28 mg/l auf 1,9-9,6 mg/l (Mittelwerte)) ließ die Wirksamkeit von biologischen Abbauprozessen erkennen. Gleichzeitig korrelierte die Anreicherung von Eisen(II) im Grundwasser in Fließrichtung von 0,15 mg/l im Anstrom auf 30-74 mg/l im Abstrom der Kontamination mit den steigenden Substratgehalten. In allen kontaminierten Messstellen wurden erhöhte Methangehalte (bis 15 mg/l) nachgewiesen, was auf einen mikrobiellen Aromatenabbau primär unter anaeroben Milieubedingungen hinwies.

Eine Ausbildung von deutlich abgrenzbaren Redoxzonen im Abstrombereich des Testfeldes war nicht erkennbar. Stattdessen wurde eine starke Überlappung der Redoxprozesse beobachtet. In den kontaminierten Messstellen wurde eine gleichzeitige Eisen(III)reduktion und Methanogenese beobachtet.

Mit diesen Daten konnte gezeigt werden, dass der gemessene Anstieg der BTEX-Konzentration im Grundwasser an der Messstelle MP2 im Sommer 2003 nicht die Folge eines eingeschränkten biologischen Abbaus war, sondern hauptsächlich auf den Einfluß des Grundwasserstandes zurückzuführen ist. Am Standort erfolgt eine Beeinflussung der Schadstoffgehalte im Grundwasser sowohl durch die Schwankungen des Grundwasserstandes als auch durch natürliche Abbau- und Rückhalteprozesse in der gesättigten Zone. Dabei war jedoch der Einfluss der Grundwasserschwankungen im Untersuchungsgebiet auf die Schadstoffkonzentrationen wesentlich stärker als der Einfluss von biologischen Abbauprozessen. Diese Schlussfolgerung konnte durch die

verhältnismäßig lange Laufzeit der Untersuchungen erarbeitet werden, da somit kurzfristige Zu- und Abnahmen der BTEX-Konzentrationen im Grundwasser gut evaluiert wurden. Für eine vollständige Klärung und detaillierte Auflösung der biochemischen Prozesse bedarf es jedoch weiterer Untersuchungen zur Lokalisierung der Kontamination in der ungesättigten Bodenzone im Abstrombereich.

Der biologische Abbau von Kohlenwasserstoffen lässt sich weiterhin anhand von aromatischen Karbonsäuren im Grundwasser nachweisen. Dieser Nachweis von organischen Säuren, welche eindeutig durch biologischen Umsatz von BTEX entstehen, wird im Rahmen von MNA-Untersuchungen immer häufiger als wichtiger Indikator für natürliche Abbauprozesse verwendet. Durch die Bestimmung von metabolischen Einzelverbindungen, wie Benzoesäure und Benzylbernsteinsäure, sowie deren methylierte Homologverbindungen, können Rückschlüsse auf die Ausgangsstoffe im kontaminierten Grundwasserleiter gezogen werden.

Die Analytik von organischen Säuren im Grundwasser erfolgte durch Gaschromatographie gekoppelt mit Massenspektrometrie (GC/MS) kombiniert mit einem Extraktionsverfahren, wie Flüssig-Flüssig Extraktion oder der Festphasenextraktion. Zu Beginn dieser Arbeit wurden die Metabolite mithilfe der Flüssig-Flüssig Extraktion aus dem Grundwasser extrahiert. Da diese Methode jedoch sehr zeitaufwendig und durch einen hohen Verbrauch an Chemikalien gekennzeichnet ist, wurde der Einsatz einer Festphasenextraktion (Solid-Phase-Extraction (SPE)) untersucht und entwickelt. Hierzu wurden verschiedene Sorbentien in unterschiedlichen Kartuschengrößen von mehreren Herstellern getestet. Nach ersten Versuchen zur Bestimmung der Wiederfindungsraten des Surrogatstandards Chlorphenylessigsäure aus BTEX-kontaminierten Grundwasserproben wurden die SPE-Kartuschen Supelclean^{TM} ENVI 18^{TM} der Fa. SUPELCO, Bellafonte, PA USA ausgewählt. Weitere Versuche wurden mit Leitungswasser und unkontaminiertem Grundwasser mit den Standardreinsubstanzen Chlorphenylessigsäure, 4(Trifluoromethyl)-Hydrozimtsäure, Benzoesäure (BA) und Benzylbernsteinsäure (BSA) durchgeführt.

Das entwickelte Verfahren der Festphasenextraktion wird im Folgenden kurz erläutert:

500 ml einer Grundwasserprobe werden abgemessen und mit konz. Salzsäure (HCl) auf einen pH-Wert von <2 eingestellt. Für die Bestimmung der Wiederfindungsraten werden die Proben mit jeweils 20 µl aus 1 mg/ml - Lösungen der Surrogatstandards Chlorphenylessigsäure und 4(Trifluoromethyl)-Hydrozimtsäure versetzt. Die Extraktion wird an einer Anreicherungseinheit mit max. 20 Adaptern für Festphasenkartuschen mithilfe einer Vakuumpumpe durchgeführt. Vor Probenaufgabe werden die Kartuschen in drei Schritten mithilfe von Aceton, Methanol und destilliertem Wasser (pH < 2) konditioniert. Zur Extraktion werden die Kartuschen über Adapter und Teflonschläuchen mit den Wasserproben verbunden und die Proben im Durchfluss bei Unterdruck durch die konditionierten Sorbentien der Kartuschen gespült. Nach dem Durchlaufen der Wasserproben werden die Sorbentien unter Stickstoffstrom getrocknet. Die Elution der organischen Säuren erfolgt durch Methanol mithilfe von Vakuumdruck. Das in Gewindeflaschen aufgefangene Eluat wird bis zur Trockene eingedampft und mit Trimethylsulfoniumhydroxid (TMSH) derivatisiert. Vor der Messung wird das Alkan Squalan zugegeben, welcher als interner Standard für die Quantifizierung der einzelnen Komponenten aus den Extrakten dient. Für die gaschromatische Auftrennung der Extrakte und die Detektion der einzelnen Substanzen wird ein GC/MS vom Typ Thermo Quest MD 800 mit einem Autosampler von FISONS AS 800 eingesetzt. Mit Wiederfindungsraten für Benzoesäure (BA) und Benzylbernsteinsäure (BSA) zwischen 75-97% konnten bei den Experimenten gute Ergebnisse erzielt werden. Die Nachweisgrenzen der Methode liegen für BA bei 0,7 µg/l und für BSA bei 0,8 µg/l.

Der entscheidende Vorteil der Festphasen-Extraktion gegenüber der Flüssig/Flüssig-Extraktion besteht in der Einsparung großer Mengen organischer Lösungsmittel. Zudem findet bei der Festphasen-Extraktion von 1 l Wasserprobe und einer Eluatmenge von 1 ml bereits eine Aufkonzentrierung der Substanzen um den Faktor 1000 statt. Dadurch können niedrige Nachweisgrenzen erzielt werden. Weiterhin kann der Zeitaufwand der Probenaufbereitung erheblich vermindert werden, da bei der Festphasen-Extraktion mehrere Grundwasserproben (bis max. 20) gleichzeitig extrahiert werden können.

Am Untersuchungsstandort zeigte die Messstelle im Anstrom und in den tiefen Schichten des Grundwasserleiters nur geringe Gehalte von BA, welche natürlichen Hintergrundkonzentrationen entsprechen (bis zu 5 µg/l). Benzoesäure kann ebenfalls durch natürliche Quellen und aus anthropogenen Quellen in die Umwelt eingetragen werden. Jedoch traten besonders in den oberen Schichten des Grundwasserleiters im Bereich des Testfeldes und im nahen Abstrombereich erhöhte Konzentrationen der C_1-C_3-BA's auf, welche mit erhöhten Substratgehalten (BTEX) im Grundwasser einhergingen. Eine direkte Korrelation zwischen BTEX und Karbonsäuren konnte nicht herausgestellt werden. An der Messstelle im Testfeld, wo durchschnittliche BTEX-Konzentrationen von 532 µg/l gemessen wurden, betrugen die durchschnittlichen Konzentrationen der C_2-BA's (Summe der Isomere) 142 µg/l und der C_3-BA's (Summe der Isomere) 91 µg/l. An den Messstellen im Abstrom wurden deutlich höhere BTEX-Konzentrationen gemessen (bis 3300 µg/l; Mittelwert MP5-f), jedoch weitaus geringere Metabolitenkonzentrationen registriert (C_2-BA=20 µg/l, C_3-BA=16 µg/l an der GWM MP5-f; Summe der Isomere; Mittelwerte). Vielmehr wurde eine Abhängigkeit zwischen den methylierten Karbonsäuren und dem schwankenden Grundwasserständen festgestellt. Mit steigendem Grundwasserspiegel stiegen die Konzentrationen der C_2- und C_3-Benzoesäuren an und sanken entsprechend wieder in trockeneren Perioden. Es wird angenommen, dass bei steigendem Grundwasserspiegel durch den erhöhten Eintrag von Nährstoffen, wie Nitrat oder Sulfat, der biologische Abbau von BTEX im Grundwasser angeregt wird. Die Metabolite konnten im gesamten Monitoringzeitraum in den kontaminierten GWM nachgewiesen werden.

Um die Erkenntnisse der im Boden ablaufenden Abbauprozesse zu erweitern, wurde im Rahmen der bearbeiteten Thematik der Zusammenhang der Fraktionierung von Eisenisotopen während der Reduktion von gebundenem Eisen(III) im Sediment zu wasserlöslichem Eisen(II) im Grundwasser untersucht. Dazu wurden einmalig Grundwasserproben der Messstellen B8, MP1, MP2, MP4 und MP5 (jeweils flach verfilterte Messstellen) und Bodenproben der Bohrkerne B3 und B5 bezüglich der Eisenisotope untersucht. Für die Bestimmung der Fe-Isotopenverhältnisse kam die hoch massenauflösende MC-ICP-MS der Universität Frankfurt zum Einsatz. Die Proben wurden im Reinraumlabor vorbereitet und mindestens zweimal

gemessen.

Im unkontaminiertem Grundwasser an der Messstelle MP1 wurde ein δ^{56}Fe-Wert von 0,01‰ bestimmt. Die δ^{56}Fe-Werte der kontaminierten Grundwasserproben, welche stromabwärts des Testfeldes genommen wurden, zeigten dagegen Deltawerte um -0.20‰ und sind somit isotopisch leichter.

Die Bodenprofile wurden jeweils einmal pro Meter beprobt (8 Proben je Bohrung) und zeigten Deltawerte zwischen 0,02‰ und 0,25‰ an, welche isotopisch schwerer als das kontaminierte, das Sediment umgebende Grundwasser waren. Die isotopische Zusammensetzung der Bodenproben überlappte mit der Zusammensetzung der unkontaminierten Grundwasserprobe. Der maximale Unterschied in den Deltawerten von δ^{56}Fe zwischen Boden und Grundwasser betrug 0.46‰. Diese Ergebnisse zeigten eine deutliche Isotopenfraktionierung zwischen Bodenproben und kontaminiertem Grundwasser, welches stromabwärts des Testfeldes entnommen wurde.

Die genauen Ursachen für diese Isotopenfraktionierung konnten im Rahmen dieser Arbeit nicht untersucht werden. Aufgrund von Literaturstudien konnten verschiedene Ansätze in Betracht gezogen werden. Jedoch wurde bei allen Arbeiten in diesem Feld herausgestellt, dass es durch den biologischen Umsatz von Schadstoffen durch eisenreduzierende Bakterien es zu einer Fraktionierung im Grundwasser und Sediment kommen kann. Diese wurde vorallem in verschiedenen Labor- und in-situ Studien zum Abbau von Kohlenwasserstoffen beobachtet. Wie stark jedoch die Isotopie durch eine sofortige Rückführung des gelösten Eisens aus dem Grundwasser in Monosulfide beeinflusst wird, ist noch nicht ganz geklärt. Weiterhin wurden in der Literatur Beispiele angeführt, welche auf die Rolle von organischen Liganden in der Fraktionierung hinweisen. Liganden erhöhen die Bioverfügbarkeit von mineralisch gebundenem Eisen und beeinflussen somit die Fe(II)-Konzentrationen im Grundwasser.

Die Bestimmung der Eisenisotopie in kontaminiertem Boden und Grundwasser birgt die Möglichkeit den tatsächlichen Umsatz von Eisen und den mikrobiologischen Abbaupfad von Schadstoffen genauer zu bestimmen als dies mit den bisher eingesetzten Verfahren möglich war. Innerhalb der wenigen momentan verfügbaren Methoden, welche dem Nachweis einer biologischen Sanierung im Untergrund dienen, bietet der Nachweis von Isotopenfraktionierungen eine vielversprechende Möglichkeit

mit unterschiedlichen Anwendungen.

Die Bewertung und Anwendung von biologischen Abbauprozessen in kontaminierten Grundwasserleitern erhielt in den vergangenen Jahren eine immer stärkere Bedeutung, was zu einem großen Teil der Akzeptanz von MNA und von intrinsischen Sanierungsmethoden zugeschrieben werden kann. Jedoch besteht bezüglich dieser Thematik noch ein deutlicher Forschungsbedarf.

Organisation of the thesis

This thesis is based on the preparation of manuscripts for publication in international journals. Obtained data from the monitoring site 'Schäferhof–Süd' were evaluated and presented in a respective different context, all within the main topic of 'Anaerobic biodegradation of BTEX in groundwater'. Because of that you will find especially in the subsections 'Introduction' of the several chapters similarities due to the background of the main topic. This is due to the presented form of the chapters as one complete manuscript. Only small parts, like 'Field description' for instance is presented seperately in chapter 2 for all chapters at the beginning of the thesis.

Chapter 3 describes the analysis of metabolites which are formed during biodegradation of BTEX and PAHs in groundwater. A solid-phase extraction method was developed for optimising the analytic process.

A detailed study of the terminal electron acceptor processes at the monitoring site is presented in chapter 4. The variations in the concentration levels of methane, ferrous iron, sulphate, nitrate and oxygen in the groundwater samples indicate clearly biological mediated processes.

Chapter 5 shows the significance of groundwater level fluctuations regarding the BTEX concentrations in groundwater at a residual contamination in the unsaturated zone at a contaminated site. The content of this chapter has been published in german language with the title "Langzeituntersuchungen zum Einfluss von Grundwasserschwankungen auf die BTEX-Konzentration im Grundwasser" in Grundwasser, 12:125-132 (2007).

A new approach in the context of BTEX degradation is the investigation of Fe isotopes in groundwater and sediment. First investigation results are presented in chapter 6.

In the appendix the compiled data are listed of the analytical results for groundwater sampling.

Abbreviations

Deutsche Abkürzungen

BBR	Bundesamt für Bauwesen und Raumordnung
BTEX	Benzol, Toluol, Ethylbenzol, Isomere des Xylols
FKZ	Förderkennzeichen
GWM	Grundwassermessstelle
GOK	Geländeoberkante
MKW	Mineralölkohlenwasserstoffe
PAK	Polyzyklische Aromatische Kohlenwasserstoffe
TL	Tanklager
UBA	Umweltbundesamt

English Abbrevations

AAS	Atomic Asorption Spectroscopy
ASL	Above See Level
BA	Benzoic Acid
bgs	below ground surface
BSA	Benzyl-Succinic Acid
BTEX	Benzene, Toluene, Ethylbenzene and the three isomers of Xylene
EPA	Environmental Protection Agency
GC/MS	Gas Chromatography/Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
MC-ICP-MS	Multi Collector-Inductively Coupled Plasma-Mass Spectrometry
MDL	Method Detection Limit
MNA	Monitored Natural Attenuation
MTBE	Methyl tert.- Buthyl Ether
NA	Natural Attenuation
PAHs	Polycyclic Aromatic Hydrocarbons
RSD	Relative Standard Deviation
SD	Standard Deviation
SPE	solid-phase extraction
TEA	Terminal Electron Acceptor
TEAPs	Terminal Electron Acceptor Processes
TMSH	Trimethyl Sulfonium Hydroxide
4TFM hydro -cinnamic acid	4(Trifluoromethyl)hydro-cinnamic acid
UST	Underground Storage Tank
TIC	Total Ion Chromatogram
RR	Relative Recovery
RT	Retention Time

1 Introduction

As a result of human activities and accidents, organic substances such as petroleum and their derived products leak into the subsurface and this on a worldwide basis. These chemicals are of fundamental importance for our industrialised civilisation and are of ubiquitous use due to their dominant role not only as a raw material for fuel production but also in the wide range of chemical synthesis products available.

Benzene, toluene, ethylbenzene, the three xylene isomers (known collectively as BTEX) are natural constituents of crude oil but are usually synthetised from other compounds present in petroleum. Today, these monoaromatic hydrocarbons are among the most common pollutants of groundwater thereby posing a direct or indirect threat to the environment and subsequently posing a threat to the world´s scarce drinking water resources due to their water solubility (see tab. 1) and toxicity (Huff et al., 1988; Maltoni et al., 1997; Röling & van Verseveld, 2002; An, 2004; Kao et al., 2006).

Table 1: Data on chemical properties of BTEX (GESTIS, 2009).

	molecular formula	molecular weight [g/mol]	density [g/cm^3]	solubility in water at 20°C [g/l]
benzene	C_6H_6	78.11	0.88	1.77
toluene	C_7H_8	92.14	0.87	0.47
ethylbenzene	C_8H_{10}	106.17	0.87	0.14
o,m,p-xylene	C_8H_{10}	106.17	0.86-0.87	0.16-0.2

The ubiquitous use of petroleum inevitably leads to the input of hydrocarbons into the environment. Punctual contamination plays a predominant role arising from transport, storage sites and petroleum refining products related spills. Drastic groundwater contaminations caused by gasoline, aviation fuel and other refined petroleum derivatives occur on a worldwide basis in particular at underground motor fuel storage tanks and at former military sites (e.g. U.S. EPA, 1986; Beller, 1995; Wiedemeier et al., 1999; Martus, 2002; Andreoni & Gianfreda, 2007).

Nearly 2,8% of the area in Germany was used by the military until 1990 (Agel & Löbel, 1999). Always the half of the military sites were disused in the meantime and

above 300.000 potentially contaminated areas were recorded. A decontamination of the subsurface and groundwater of these areas is required (Hettwer, 2006).

On the one hand it is very difficult to remediate by active measures a subsurface environment. On the other hand in most of the cases it is too expensive. Conventional pump-and-treat technologies may contain and control subsurface contaminant plumes yet these techniques are limited in their effectiveness in remediating groundwater pollution. This is for instance due to the complex and inhomogeneous nature of most aquifers and sorption/desorption processes of the contaminant onto solid media. Many current pump-and-treat systems will therefore continue to run indefinitely and new innovative technologies capable of destroying contaminants in situ, such as bioremediation are widely sought. New technologies are envisaged to reduce equipment, investment, operating costs and risks to public health and safety.

At many contaminated sites, the subsurface is able to attenuate pollutants thus potentially lowering the costs of remediation. The toxicity, mass and/or mobility of the contaminants can be reduced without human intervention when suitable conditions prevail. Based on this fact, the concept of natural attenuation (NA) or intrinsic bioremediation was drafted in the USA. NA is the main method for monoaromatic degradation and results indicate that up to 90 % of BTEX removal by this approach can be attributed to the intrinsic biodegradation process (Kao & Prosser, 2001). Intrinsic bioremediation is an environmental site management approach that relies on naturally occuring microbial processes for petroleum hydrocarbon removal from groundwater (Kao & Prosser, 2001; Maurer & Rittmann, 2004; Reinhard et al., 2005; Kao et al., 2006). The term monitored natural attenuation (MNA) was introduced in the context of control and for such related processes and is defined by the U.S.EPA (1999) as follows:

> *The term monitored natural attenuation, [..] refers to the reliance on natural attenuation processes (within the context of a carefully controlled and monitored site cleanup approach) to achieve site-specific remediation objectives within a time frame that is reasonable compared to that offered by other more active methods. The "natural attenuation processes" that are at work in such a remediation approach include a variety of physical, chemical, or biological processes that, under favorable conditions, act without human intervention to reduce the mass,*

> *toxity, mobility, volume, or concentration of contaminants in soil or groundwater. These in-situ processes include biodegradation; dispersion; dilution; sorption; volatilisation; radioactive decay; and chemical or biological stabilisation, transformation, or destruction of contaminants.*

Microorganisms are the principal mediators for natural attenuation of many pollutants (Hollinger et al., 1997; Christensen et al., 2001; Schulze & Thiem, 2004; Nikolova & Nenov, 2005). They transform or mineralise pollutants thereby decreasing their masses and toxicities in contrast to most other processes of natural attenuation. Reliance on intrinsic bioremediation require methods to monitor the process. Such chemical methods are based on measurements of changes of contaminant concentrations of metabolic end products and/or co-reactants along a flow path allowing rapid verification of intrinsic bioremediation (Röling & van Verseveld, 2002). Changes in the concentrations of electron acceptors for instance, indicate the occurrence of intrinsic bioremediation (Chakraborty & Coates, 2004). Also the presence of intermediary metabolites provides information on in situ degradation of specific compounds when an unequivocal and unique biochemical link with the parent compound exists, when no other sources for the particular metabolite are available and when the released product exhibits biochemical and chemical stability under in situ conditions. Preferably, metabolites should be a intermediate product of mineralization rather than a product of cometabolism (Beller, 2000).

As outlined above, a chemical analysis is of major importance in understanding microbial processes associated with natural attenuation in providing evidence and in detecting intrinsic bioremediation and likewise in the assessment of the potential of these processes in the environment.

The present thesis will make a contribution in this area of research. With reference to the Umweltbundesamt (UBA) research project "Langzeituntersuchungen zu den Möglichkeiten und Grenzen der Nutzung natürlicher Selbstreinigungsprozesse für ausgewählte Schadstoffe am Beispiel kontaminierter militärischer Liegenschaften (FKZ 298 76 712 /02)", comprehensive field investigations on BTEX-contaminated sediment and groundwater were carried out at a former military site named Schäfer-Süd. The project was a colaboration of the Umweltbundesamt, the Zentrum für

Umweltforschung und Umwelttechnologie of the University of Bremen, the University of Frankfurt/Main and the alphacon GmbH from Ganderkesee.

The required aims of the presented study were the investigations at the site Schäferhof-Süd to identify the distribution of redox-sensitive groundwater constituents in the aquifer, identify governing redox environments within the contaminated grounwater and thereby provide evidence of biological mediated BTEX degradation. The analysis of organic acids generated as metabolic by-products during biodegradation of BTEX and PAHs under anaerobic conditions were furthermore focus of this study. A SPE method was developed and validated in the analysis of metabolites in BTEX and PAHs-contaminated groundwater samples using GC-MS.

2 Field description

2.1 Location and geology

The study area Schäferhof-Süd is located southwest of Nienburg (Nienburg/Weser, Niedersachsen in Germany). Over a monitoring period of three years, investigations were carried out evaluating NA processes in the unsaturated and saturated zone at a former military underground storage tank (UST) site. On this site a gasoline filling station and a diesel fuel storehouse was also located. The UST site was shown to contain a residual contamination in the subsurface, high alkylated benzenes and aliphatic petroleum-derived hydrocarbons preferentially in the capillary fringe of the sediment and in the groundwater. Figure 1 depicts the location of the UST Schäferhof Süd.

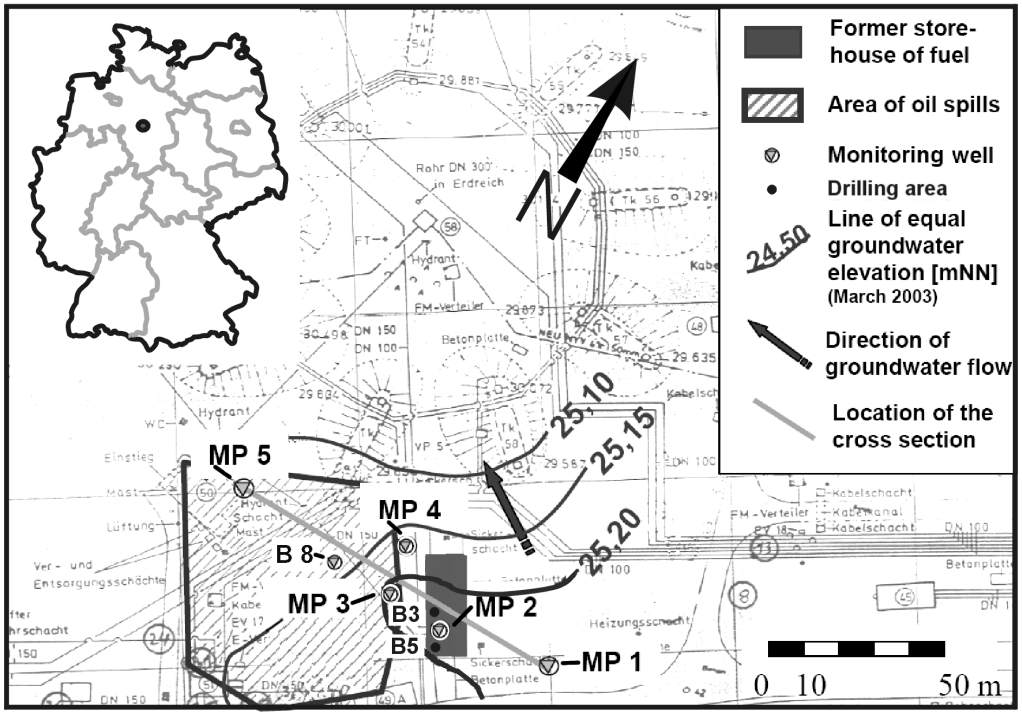

Figure 1: Location of the study site UST Schäferhof Süd.

Six observation wells were sampled at regular intervals in order to investigate the extent of contaminated groundwater, the terminal electron acceptor processes

(TEAPs) and the metabolites of BTEX degradation in the groundwater. One of these wells (B8) is screened from 4 m below ground surface (bgs) to 12 m bgs. The five remaining monitoring wells (MP1-MP5) were drilled as double level monitoring wells with one well screening from 4 to 7 m bgs (upper section, MP1-f-MP5-f) and one well screening from 7 to 10 m bgs (lower section, MP1-t-MP5-t). The wells were positioned along a transect in order to incorporate upstream, source and downstream areas of presumed source (former diesel fuel storehouse, see fig. 2).

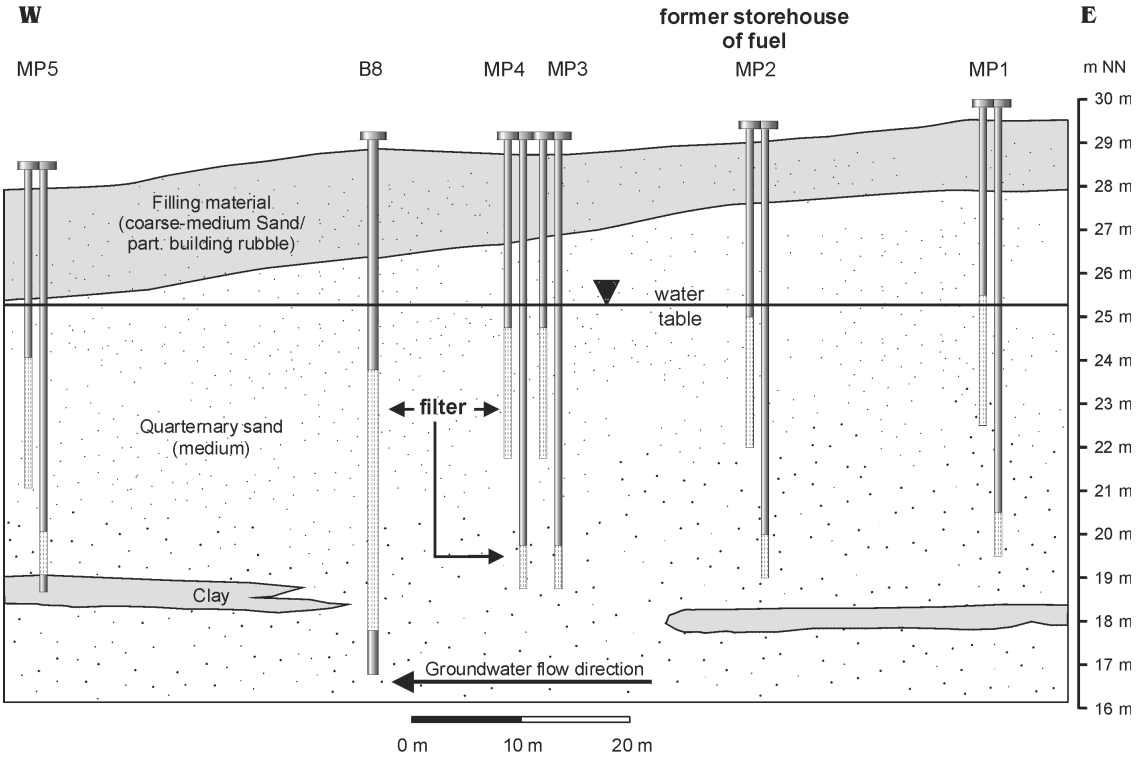

Figure 2: Schematic cross section of the study area illustrating the hydrogeology within 14 m of the land surface. Refer to Figure 1 for location. The positions of the observation wells, the filter range in the wells and the location of the former fuel storehouse are shown. The regional water table during sampling in March 2003 is indicated.

The site is geologically situated in the Schessinghausener Graben. This regional rift structure strikes in a North–South direction, starting at the salt dome structures Husum and Schessinghausen, and is transsected almost perpendicularly by the river Weser. Cretaceous and Tertiary shales, marls and sand stones are deposited on a

1.000 m Upper Jurassic sediment sequence, followed by Quarternary sediments of a different thickness (Voss, 1991).

Fine to coarse grained Quarternary sands with interbedded discontinued clay lenses are predominant in the Mittelweser valley, where the former military site is located. The Quarternary sands are represented by an accumulation of Holocene inland dunes, Weichsel glacial terraces and Saale glacial sediments. These units constitute the upper free aquifer with a thickness of 50-70 m. The depth of the groundwater level varies between 4 and 7 m. The hydraulic conductivity of the sediments is characterised by a value of $1 \cdot 10^{-4}$ m/s. The general groundwater flow is westwards in the direction towards the river Weser (fig. 1).

2.2 History of the former UST Schäferhof-Süd

UST Schäferhof-Süd and the fuel storehouse were erected in 1935 as a gasoline depot. It was in operation until 1945 used by the Wirtschaftliche Forschungsgemeinschaft mbH (Wifo) and bombed while the Second World War in 1944. The use of the depot as a filling station came to an end in 1945. The British Armed Forces adopted and reconstructed the site, using the site again as a gasoline depot. In 1976 the site was acquired by the Deutsche Bundeswehr and since then unused.

In 1990, the underground fuel storage tanks and the pumping stations were removed. During clean-up operations in 1995-2001, the fuel and pumping stations tanks were removed and buildings demolished.

The excavation work laid a sound basis in a successful remediation of the remaining subsurface contamination. Until 2001 no short-term usage of this area was planned which allowed to use the area for a long-term research project initiated and supervised by the German Umweltbundesamt (UBA) (Hettwer et al., 2006). In particular, the area of the former filling station and the diesel fuel storehouse were selected as an appropriate test plot at the UST site Schäferhof Süd.

3 Analysis of metabolites from anaerobic BTEX and PAH degradation in groundwater by solid-phase extraction (SPE) coupled with gas chromatography-mass spectrometry (GC/MS)

Abstract

Biodegradation of aromatic hydrocarbons under anaerobic conditions in the subsurface at contaminated sites is characterised by generation of metabolites. From the monoaromatic hydrocarbons benzene, toluene, ethylbenzene and the three isomers of xylene (BTEX) metabolites such as benzoic acid (BA) and the methylated homologs and C_1-and C_2-benzyl-succinic acids (BSA) are generated as intermediates. 2-naphthoic acid, isomers of tetrahydronaphthoic acid, octahydronaphthoic acid and naphtyl-2-methylsuccinic acid were identified as metabolites from naphthalene. Additionally several metabolites from three ring polycyclic aromatic hydrocarbons (PAHs) have been reported in literature. A solid-phase extraction method based on octadecyl-bonded silica sorbent has been developed to concentrate such metabolite compounds from water samples followed by derivatisation and gas chromatography/mass spectrometry (GC/MS) of the extracts. Recovery experiments with authentic standards of BA and BSA were performed with groundwater and tap water samples. The recovery rates for BA and BSA ranged between 75% and 97%. The method detection limits for BA was 0.7 µg/l and 0.8 µg/l for BSA.

3.1 Introduction

Monoaromatic hydrocarbons, including benzene, toluene, ethylbenzene, the three xylene isomers (BTEX), and polycyclic aromatic hydrocarbons (PAHs) are today among the most common pollutants of groundwater. BTEX and PAH pose great environmental and regulatory concern due to their water solubility and toxicity (Maltoni et al., 1997; Huff et al., 1988; An, 2004; Kao et al., 2006) and are therefore generally included in groundwater monitoring programmes (e.g. Wiedemeier et al., 1999). BTEX are constituents of gasoline and jet fuel. Leakages of underground storage tanks containing these petroleum products or surface spill accidents belong to the prevalent challenges of sediment and groundwater cleanup operations. Moreover, BTEX are generated during coal pyrolysis and are therefore common pollutants in sediment and groundwater at coking plants and former gas works.

Similar to BTEX, polycyclic aromatic hydrocarbons (PAHs) are also products of coal pyrolysis and the predominant constituents of coal tar. PAHs are sometimes present in high concentrations in the subsurface of coking plants and former gas works and were also distributed into the environment at high concentration levels by technical use of coal tar (WHO, 2004). The PAH homologs with four- to six-member rings are known or suspected carcinogens (Yang & Silverman, 1988).

Natural attenuation (NA) is increasingly accepted as one option for cleanup of BTEX and PAHs contaminated aquifers, provided that the effectiveness of natural degradation processes of the hydrocarbons has been proven at a contaminated site (Wiedemeier et al., 1999). In many laboratory and field studies the degradation of aromatic hydrocarbons is shown under a variety of terminal electron-accepting conditions (e.g. Spormann & Widdel, 2000; Phelps & Young, 2001; Meckenstock et al., 2004b; Schreiber et al., 2004; Roychoudhury & Merrett, 2006). The biochemical processes during biodegradation are investigated increasingly in detail and new methods for analysis are still sought-after (e.g. Yang & Silverman, 1988; Beller, 1995; Spormann & Widdel, 2000; Phelps & Young, 2001; Meckenstock et al., 2004b; Chen et al., 2008).

Parameters derived from the analysis of electron acceptors or metabolic by-products can act as indicators for biologically mediated degradation. The naturally occurring electron acceptors are dissolved oxygen, nitrate, ferric iron, sulphate and carbon dioxide, which are consumed during microbial metabolism of organic con-

taminants. The role of electron acceptors in biodegradation is described in detail elsewhere (e.g. Yang & Silverman, 1988; Cozzarelli et al., 1995; Heider et al., 1999; Wiedemeier et al., 1999; Spormann & Widdel, 2000). Due to the low solubility of oxygen in groundwater, aquifers contaminated with hydrocarbons usually become anoxic with a redox gradient along the flow path and the major fraction of contaminants is degraded in the anoxic zones of the plume (Beller, 1995; Cozzarelli et al., 1995; Wisotzky & Eckert, 1997; Heider et al., 1999; Beller, 2000; Spormann & Widdel, 2000; Phelps & Young, 2001; Namocatcat et al., 2003; Meckenstock et al., 2004b).

Because of the low water solubility of PAHs compared to BTEX, the bioavailability of the compounds is reduced with increasing numbers of aromatic rings. Therefore, PAHs containing up to three rings can be transformed readily, but higher condensed compounds can only be metabolized in the presence of smaller ones or solvents like BTEX, which enhance the solubility (Coates et al., 1997; Geller et al., 2001). However, anaerobic biodegradation of PAHs under nitrate- and sulphate-reducing conditions is only significant for lower condensed compounds like naphthalene, methylnaphthalenes, phenanthrene, fluorene, and with some restrictions for fluoranthene and acenaphthene (Meckenstock et al., 2000; Geller et al., 2001; Meckenstock et al., 2004b).

In the focus of the presented study is the analysis of organic acids generated as metabolic by-products during biodegradation of BTEX and PAHs under anaerobic conditions. In groundwater at former gas works and coking plants BTEX, PAHs of lower molecular weight and abundant metabolites of these hydrocarbons were detected by Annweiler et al. (2001); Griebler et al. (2004) as well as by Gödeke et al. (2006). Most of these metabolites are organic acids like benzoic acid (BA) or benzylsuccinic acid (BSA) and the methylated homologs. These compounds are used as indicators of degradation processes for BTEX under anaerobic conditions (Sembiring & Winter, 1989; Beller, 1995; Elshahed et al., 2001; Beller, 2002). Among these compounds benzoic acid, although an intermediate of toluene degradation (Sembiring & Winter, 1989) is not an appropriate biogeochemical indicator for benzene degradation because of its widespread use in commercial products and its occurrence as intermediate during the anaerobic metabolism of several other aromatic compounds (Beller, 1995).

The detection of alkylated benzoic acid, however, indicates biodegradation of pollutants such as kerosene or gasoline in groundwater. Aromatic acids are formed by the anaerobic hydroxylation of the methyl group. As an intermediate step, derivatives of benzylsuccinate are generated, like exemplarily shown for anaerobic toluene degradation by Biegert et al. (1996); Alumbaugh et al. (2004). Methylbenzoic acid is an intermediate of the degradation path of C_2-benzene. Analogously are the parent hydrocarbonates for the C_2- and C_3-benzoic acids C_3- and C_4-benzenes, respectively (Beller, 2000; Namocatcat et al., 2003). In addition to the oxidation of methyl groups organic acids can also be generated by carboxylation of the aromatic system as the initial degradation step, like shown for naphthalene and phenanthrene (Zhang & Young, 1997; Meckenstock et al., 2000).

Furthermore, benzylsuccinic acid and methylbenzylsuccinic acid isomers are proposed as distinct indicators of anaerobic toluene and xylene metabolism. These succinic acids have no commercial or industrial use and an unequivocal and unique relationship to their parent hydrocarbons is possible (Beller, 2002; Reusser et al., 2002; Namocatcat et al., 2003; Gödeke et al., 2006).

Other key substrates to proof biologically mediated degradation of hydrocarbons in groundwater are aliphatic fatty acids, which are constituents of cellular membranes of microorganisms and plants. Increased concentrations of tetradecanoic acid, hexadecanoic acid and octadecanoic acid in groundwater can be an additional indicator for biological activity (Zhang & Young, 1997).

In several previous laboratory and field investigations analytical approaches for the detection of aromatic acids were developed. Liquid-liquid extraction (LLE) coupled with gas chromatography-mass spectrometry (GC-MS) (e.g. Evans et al., 1992; Cozzarelli et al., 1994; Beller, 1995; Cozzarelli et al., 1995; Schmitt et al., 1996; Gieg et al., 1999; Meckenstock et al., 2000; Martus & Püttmann, 2003) or liquid chromatography-tandem mass spectrometry (LC-MS-MS) (Beller, 2002) were mostly used.

In recent years, a new approach for the extraction of organic acids from groundwater samples has been developed. Reusser & Field (2002) developed a solid-phase extraction (SPE) method coupled with GC-MS for analyses of BSA and methyl-BSA compounds in BTEX-contaminated groundwater samples. Alumbaugh et al. (2004) have positively validated a SPE method coupled with LC-MS-MS.

The purpose of this study is to develop and to validate a SPE method for a comprehensive analysis of metabolites in BTEX and PAHs-contaminated groundwater samples using GC-MS of derivatised organic acids. Benzoic acid and methylated homologs (C_1-C_3), C_1-and C_2-benzylsuccinic acid, 2-naphthoic acid, isomers of tetrahydronaphthoic acid, methylnaphthoic acid and naphtyl-2-methylsuccinic acid, as well the fatty acids tetradecanoic acid, hexadecanoic acid and octadecanoic acid were analysed.

3.2 Experimental

3.2.1 Chemicals and equipment

DL-benzylsuccinic acid (BSA, 99% purity) is purchased from Sigma-Aldrich (Steinheim, Germany) and benzoic acid (BA, 99% purity) from Merck (Darmstadt, Germany). Squalane (99% purity) was obtained from Fluka (Buchs, Switzerland) and 4(trifluoromethyl)hydro-cinnamic acid (4TFM hydro-cinnamic acid, 95% purity) and 4-chlorophenylacetic acid (99% purity) from Sigma-Aldrich (Steinheim, Germany). Squalane was used as internal standard, 4TFM hydro-cinnamic acid and 4-chlorophenylacetic acid were used as surrogate standards. Standard solutions of BSA, BA, 4TFM hydro-cinnamic acid and 4-chlorophenylacetic acid were prepared in acetonitrile at 1 mg/ml. The internal standard was prepared in hexane at 1 µg/µl. Methanol (99% purity, distilled), acetone (99% purity), hexane (99,5% purity) and acetonitrile (HPLC-grade) were purchased from AppliChem (Darmstadt, Germany). Ethyl acetate (HPLC-grade) was obtained from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid (conc.) was obtained from Merck (Darmstadt, Germany). For derivatisation trimethyl sulfonium hydroxide (TMSH) from Fluka (Buchs, Switzerland) was used.

SPE was performed using a 20-fold vacuum extraction box (Vac Elut 20 from Varian). SupelcleanTM ENVI^{TM} 18 cartridges with a volume of 3 ml were obtained from Supelco (Bellafonte, PA USA) filled with the sorbent octadecyl-bonded silica (C18).

3.2.2 Field sites

Groundwater samples contaminated with BTEX were taken from the former military site Schäferhof-Süd near Nienburg (Niedersachsen, Germany). Between 2001 and 2004, long-term measurements for natural attenuation of monoaromatic hydrocarbons in sediment and groundwater were performed at a former gasoline filling station and a storehouse for fuels (see chap. 2). The locality contains a large residual contamination of BTEX and higher alkylated benzenes in the subsurface. For investigations of PAHs, contaminated groundwater samples were collected separately from a site with PAH contamination in groundwater.

3.2.3 Analytical procedure

3.2.3.1 Sampling and storage

The groundwater samples were collected with a submersible pump (Grundfos, type MP1). The samples were taken after a minimum of three volumes of water had been removed from the wells and the parameters temperature, pH, conductivity and content of oxygen has been stabilised.

For determination of metabolites 1 l of groundwater was filled in dark glass bottles and stored at approximately 4℃. The samples were analysed within one week after sampling and were not filtered.

3.2.3.2 Solid-phase extraction

Prior to extraction, groundwater samples were warmed up to room temperature. 0.5 l of the samples was adjusted to pH 2.0 with concentrated HCl. After acidification, all samples were spiked with 20 µl of 1 mg/ml of the 4-chlorophenylacetic acid and 20 µl of 1 mg/ml of the 4TFM hydro-cinnamic acid surrogate standard.

The ENVI^{TM} 18 cartridges were placed in the vacuum manifold and preconditioned in three steps. Firstly, 2.0 ml acetone passed the cartrige by gravity and were allowed to dry. Thereafter 2.0 ml methanol was applied under vacuum and the cartridges were not allowed to fall dry until the end of extraction. Then, 2.0 ml of pH 2 deionized water was applied. Finally, the groundwater sample was filtered

through the cartridge by a negative pressure of 800 mbar. For the connection of the sample flasks and the cartridges, PTFE tubes and tube adapters were used.

After extraction, the sorbent of cartridges was dried under a slight steam of dry nitrogen. The compounds adhering to the sorbent were eluted by passing three times 660 µl aliquots of methanol which were collected in 2 ml vials (Trott/CZT, Kriftel, Germany). The extracts were afterwards dried down under an extractor hood at room temperatur.

In a next step the dry extract was weighed and the samples were redissolved in 1 ml acetonitrile. From these solutions aliquots containing approximately 1 mg dry-extract were taken and transferred to a 1.1 ml autosampler vial. The extracts were again dried down carefully, weighed and derivatised using 33 µl/mg TMSH. The sample vials were capped and the reaction was allowed for one hour while heated to 60℃. Then samples were cooled to room temperature, spiked with 5 µl of 1 µg/µl of the internal standard squalane and after addition of 300 µl acetonitrile placed into the autosampler of the GC/MS for analysis.

3.2.3.3 Gas chromatography - mass chromatography

The analyses of the derivatised components were performed with a Thermo Quest MD 800 (GC 8000 series/ MS MD 800) equipped with an Fisons AS 800 autosampler. Measurement control and data acquisition was achieved using the software MassLab 1.2. For the chromatographic separation a ID-BPX5 silica capillary column (non-polar, 5% phenylpolysilphenylene-siloxane; 30 m length by 0.25 mm i.d.) was used with helium as carrier gas. The injector was operated under splitless conditions at 280°C with a 1 µl injection volume. The program for the column temperature had an initial temperature of 40°C (held 1 min) and increased at 4°C/min to a end temperature of 300°C (held 30 min). The MS system was operated in electron impact mode with a source temperature of 220°C. The detector operated in the full scan mode, from 50 to 600 mass units. Mass chromatography (MassLab 1.2) using specific fragment ions of the methylated metabolites was applied for identification of individual components. Quantification was performed using the total ion current (TIC) and internal standard concentrations. Linear calibration curves were obtained, typically with $r^2 = 0.998$ for BA and with r^2=0,999 for BSA.

3.2.4 Sample preparation for method validation

The method was developed to quantify metabolites, as benzoic acid, methylbenzylsuccinic acid and their methylated forms of BTEX degradation. Furthermore, the method was tested on groundwater samples contaminated with PAHs. Metabolites of PAH degradation, like 2-naphthoic acid, tetrahydro-2-naphtoic acid and naphthyl-2-methyl-succinic acid were identified. The method was developed and validated on the base of the commercially available compounds BA, BSA and the standards 4TFM hydro-cinnamic acid and 4-chlorophenylacetic acid.

Two sets of spike and recovery experiments for the SPE-method were carried out. In a first step, the solid-phase procedure was tested with tap water samples spiked with 50 µg/l benzoic acid and 40 µg/l 4-chlorophenylacetic acid using the following SPE-cartidges: (1) Oasis HLB-cartridges (vinyl pyrrolidone-divinylbenzene copolymer) from Waters Corporation Milford, Mass USA, (2) Bond Elut PPL-cartridges (styrol-divinylbenzene polymer) from Varian, Harbor City, CA, USA, (3) ENVI Carb cartridges (graphitized carbon black) from Supelco, Bellafonte, PA, USA and (4) ENVI 18 (C18 polymerically bonded to silicia) from Supelco, Bellafonte, PA, USA.

Quantitative recovery from tap water samples containing BA and BSA-standards could only be obtained when all glassware was silanised. Reusser & Field (2002) have suspected that organic acids and phenols present in the groundwater samples may compete with BSA for sorption sites on the glass surface. In this study, loss of BA, BSA and 4-chlorophenylacetic acid from acidic tap water in recovery experiments with non-silanized glassware has also been observed. Due to the long contact time of two to six hours of the reagents in the tap water with the glass surfaces a pretreatment of the glassware as follows is recommended. First the glassware was put for 12h in 1N HCl. After washing with deionized water and drying in air the silanization was started by rinsing for about 15 s with dimethyldichlorosilane (DMDCS 5% in toluene, Supelco). Then the glasware was washed two times with toluene, three times with methanol, and then air dried. Glassware for the elution and the derivatisation reaction was not silanised.

In the extracts of the HLB-cartridges only very low contents of organic acids were detected. Cartridges of this type were not further used. All other cartridges proved better recoveries and were therefore applied for further tests with BTEX-

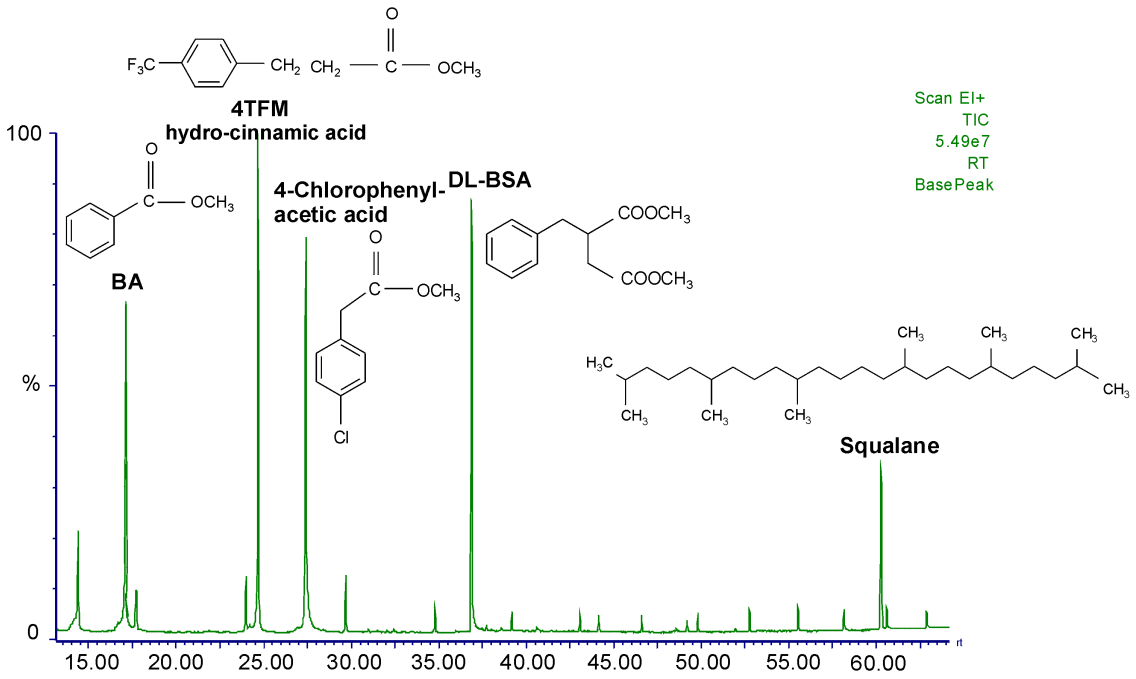

Figure 3: TIC of a tap water sample spiked with 20 µg/l of authentic standard compounds BA and DL-BSA, 20 µg/l of surrogate standards 4TFM hydro-cinnamic acid and 4-chlorophenylacetic acid and 5 µg/l internal standard squalane. BA, BSA, 4TFM hydro-cinnamic acid and 4-chlorophenylacetic acid were detected as methylesters. Retention time given in minutes.

contaminated groundwater samples from the former military site Schäferhof–Süd. All samples were taken from the same well. Samples were spiked to a final concentration of 40 µg/l 4-chlorophenylacetic acid as surrogate standard for recovery experiments. Two replicate analyses were done for every cartridge size and sample volume. One additional sample was extracted by liquid-liquid extraction (LLE) and was used as a reference. In a second set 12 uncontaminated groundwater samples from Schäferhof–Süd and 12 tap water samples were prepared. The samples were spiked with 5, 10, 50 and 100 µg of the authentic compounds BA and BSA. Additionally 20 µg surrogate standards 4-chlorophenylacetic acid and also 20 µg 4TFM hydro-cinnamic acid were added. For every concentration three replicates for groundwater and tap water, respectively, were prepared. The extraction was done by using of the ENVI 18 cartridges. Figure 3 shows a TIC of the GC/MS

analysis of a tap water extract spiked with the standard compounds and surrogate standards.

3.3 Results and Discussion

3.3.1 Recoveries and quality control

Initial experiments were focused on identifying applicable sorbents with an appropriate cartridge size for isolating metabolites generated by biological BTEX-degradation. The SPE method should substitute the time-consuming procedure of LLE. In table 2 recovery rates for 4-chlorophenylacetic acid extracted from contaminated groundwater samples which where spiked with 40 µg/l of the surrogate standard before extraction are shown. The highest recovery rates were obtained with ENVI 18 using 250 ml and 500 ml sample volume.

Table 2: Recovery rates for the surrogate standard 4-chlorophenylacetic acid for pre-concentration of different sample volumes of a groundwater sample with three sorbents in various cartridge sizes (both replicate analyses are given). For comparison, a recovery rate of 87,8% was determined with the liquid-liquid extraction (500 ml groundwater sample taken from the same well using ethylether for extraction).

Cartridge (sorbent volume)	sample volume	Recovery rate [%]
ENVI 18^{TM} (3ml)	250 ml	84,4
ENVI 18^{TM} (3ml)	250 ml	94,8
ENVI 18^{TM} (3ml)	500 ml	92.8
ENVI 18^{TM} (3ml)	500 ml	102,6
ENVI $Carb^{TM}$ (6ml)	500 ml	97,2
ENVI $Carb^{TM}$ (6ml)	500 ml	91,6
ENVI $Carb^{TM}$ (3ml)	500 ml	28,5
ENVI $Carb^{TM}$ (3ml)	500 ml	24,2
ENVI $Carb^{TM}$ (3ml)	250 ml	53,9
ENVI $Carb^{TM}$ (3ml)	250 ml	75,3
BOND Elut PPL (1ml)	250 ml	72,1
BOND Elut PPL (1ml)	250 ml	90,7

Furthermore, the ENVI Carb cartridges with a volume of 6 ml have shown good

Table 3: Recovery rates (RR) and relative standard deviations (RSD) for four different concentration levels for solid-phase extraction on ENVI 18 cartridges with groundwater and tap water samples (n=3 for every concentration)

Compound	c=5 µg/l		c=10 µg/l		c=50 µg/l		c=100 µg/l		Average	
	RR(%)	RSD(%)	RR(%)	RSD(%)	RR(%)	RSD(%)	RR(%)	RSD(%)	RR(%)	RSD(%)
groundwater										
BA	96.6	3.3	75.5	8.9	60.3	11.4	67.0	6.8	74.8	7.6
BSA	93.6	6.4	102.8	6.9	95.4	2.3	82.3	11.9	93.5	6.8
tap water										
BA	96.6	6.1	70.2	6.9	88.4	1.4	71.4	4.9	81.7	15.9
BSA	98.8	1.7	93.8	5.6	100.8	6.8	93.6	5.6	96.8	3.7

recovery rates for the surrogate standard, but fatty acids (tetradecanoic acid, hexadecanoic acid and octadecanoic acid) were detected with very low concentrations (29.0% and 30.8%, sum of the three fatty acids) compared to LLE, which was used for reference. Using ENVI 18, 57.8% and 83.5% were detected for a sample volume of 250 ml and 77.8% and 64.6% for 500 ml. These results show that by use of LLE better recovery rates can be achieved for aliphatic fatty acids. Based on this experiment the ENVI 18 cartridges were used in all subsequent experiments.

In a second set of experiments the usefulness of ENVI 18 cartridge for extracting BA and BSA at different concentrations was tested. In table 3, the recovery rates are given. The average recovery of BA from groundwater, measured relative to the squalane as internal standard, was 75% (average recovery, n=3, table 3) and from tap water 82%. The analyses provide lower recovery rates for higher concentrations of BA. The recovery rates of BSA are for both, groundwater (94%) and tap water (97%), better than for BA.

In some of the contaminated groundwater samples suspended particles were observable. Experiments using three different groundwater samples have shown that samples filtered through folded filters 597 $\frac{1}{2}$ (Ø 185 mm, Schleicher & Schüll, Dassel, Germany) provided 43 - 64% less metabolites than unfiltered samples. Apparently organic acids can accumulate on suspended particles. For this reason, groundwater samples were not filtered before extraction although flow-rates using unfiltered samples are lower during the solid phase extraction process.

Table 4: Recovery rates (RR) of BA, BSA and 4TFM hydro-cinnamic acid

Sample	Absolute 4TFM hydro-cinnamic acid RR%[a*] (RSD)	Absolute BA RR%[a*] (RSD)	Relative BA RR%[b*] (RSD)	Absolute BSA RR%[a*] (RSD)	Relative BSA RR%[b*] (RSD)
groundwater	88.9 (12.2%)	74.8 (7.6%)	92.3 (5.3%)	93.5 (6.8%)	100.2 (3.2%)
tap water	85.0 (13.4%)	81.7 (15.9%)	95.1 (6.9%)	96.8 (5.7%)	98.6 (7.2%)

[a] Absolute recovery is determined against the squalane internal standard
[b] relative recovery is determined against the 4TFM hydro-cinnamic acid surrogate standard
* average recovery from n=12

Recovery rates of 4-chlorophenylacetic acid from groundwater were 70% (13.6%) (average recovery (RSD) n=12) and from tap water 77% (13.2%). Problems using 4-chlorophenylacetic acid for quantification occurred in some samples due to peak tailing. Despite an intensive preventive maintance of the equipment, these problems reoccurred. Therefore 4TFM hydro-cinnamic acid was added as additional surrogate standard. Recovery rates for this standard were with 89% for groundwater and 85% for tap water even better than for 4-chlorophenylacetic acid, bases on 12 measurements (tab. 4). The recovery of BA, measured relative to the surrogate standard 4TFM hydro-cinnamic acid, was 92% for groundwater and 95% for tap water. The recovery of BSA relative to 4TFM hydro-cinnamic acid was 100% for groundwater and 99% for tap water, respectively (see tab. 4).

The limit of detection (LOD) was calculated after Krull & Swartz (1998) from peak areas as the standard deviation of repeated measurements. The LOD values are 0.8 µg/l for BA and 0.2 µg/l for BSA, respectively. Repeated injections (n=5) of five individual sample extracts with concentrations of 1, 2, 5, 10 and 20 µg/l BA and BSA were evaluated. Detection limits of 0.7 µg/l for BA and 0.8 µg/l for BSA were calculated using the conventional signal-to-noise method. To obtain these method detection limits, the standard deviation of the prepared groundwater and tap water samples with concentrations of 5 µg/l BA and BSA were multiplied by a factor of 3.365 (the student's t-value for a one-tailed test at the 99% confidence interval with 5 degrees of freedom (Glaser et al., 1981)). The method quantification limit of 2 µg/l can be given for BA and BSA, based on multiplying the method detection limit by a factor of three (Krull & Swartz, 1998).

3.3.2 Identification and quantification of metabolites

The identification of the metabolites was carried out by comparison of the GC retention times and of the mass spectra obtained from derivatised authentic compounds. For those compounds not available as standards, identification was based on published mass spectra. Concentrations of the analytes in all water samples were calculated by measuring the peak area of each compound relative to the peak area of squalane. For quantification of BA, BSA, 4TFM hydro-cinnamic acid and 4-chlorophenylacetic acid in spike and recovery experiments, response factors for the peak areas have been determined. These were estimated by injecting known standard concentrations of the analytes and comparing the resultant peak areas with the peak area of squalane. This allows the use of squalane for calculating the concentrations of all metabolites.

In figure 4 is shown a TIC with the mass traces of derivatised benzoic acid (m/z=136) and the methylated forms C_1- (m/z=150), C_2- (m/z=164) and C_3-benzoic acid (m/z=178) extracted from a BTEX contaminated groundwater sample. All compounds are detected as methylesters. The distribution pattern of alkylated BAs illustrates that several isomers are present. For quantification of BA a response factor of 0.52 relative to squalane was determined. Because no standard compounds were available for the methylated BAs, the identification of the compounds was achieved by mass spectrometric analysis by adding multiples of m/z=14 (the effective mass of the methyl fragment) to the mass of BA. The same response factor (0.52) was used for quantification.

In the TIC of figure 4 was calculated for BA a concentration of 3.52 µg/l and for the sum of isomers of C_1-BA 246,7 µg/l, whereas 112.3 µg/l are accounted to the third isomer at retention time of 21.17. Due to their high concentrations these isomers are dominant signals in the TIC. The sum of isomers of C_2-BA amounts to 393.8 µg/l and the sum of the isomers of C_3-BA yields 64.5 µg/l.

Figure 5 illustrates the TIC with the mass traces of two alkylated benzylsuccinic acids, extracted from the same sample shown in figure 4. Since also for methyl-BSA and C_2-BSA no authentic standards are available, BSA was used as standard compound for quantification with a response factor of 0.7 relative to squalane. For identification in the TIC the mass of the additional methyl groups was added to the mass of BSA. The mass spectra of the detected methyl-BSA were identical with

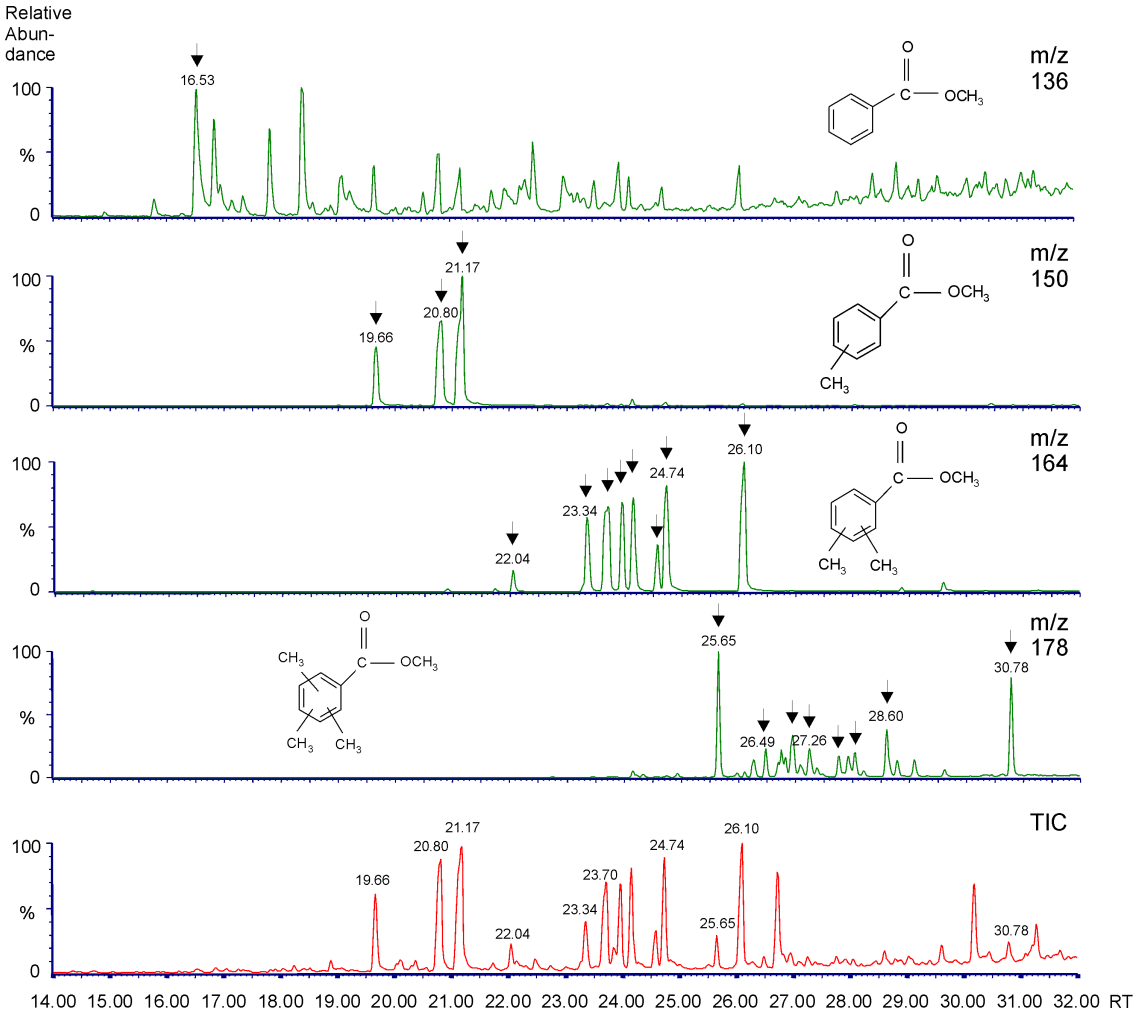

Figure 4: Distribution patterns of TMSH-derivatised aromatic acids in a total ion chromatogram with the mass traces m/z=136 for BA, m/z=150 for C_1-BA, m/z=164 for C_2-BA and m/z=178 for C_3-BA extracted from a BTEX contaminated groundwater. Compounds were detected as methylesters. Retention times are given in minutes.

mass spectra published previously by Evans et al. (1992); Beller (1995); Martus & Püttmann (2003). Martus & Püttmann (2003) have also detected higher alkylated compounds of BSA, like C_2-BSA to C_6-BSA.

Three isomers of methyl-BSA could be detected with a summarised concentration of 60.2 µg/l in this sample. For C_2-BSA four isomers were detected with a concentration of 33.1 µg/l in total. Additionally, the aliphatic fatty acids tetradecanoic acid, hexadecanoic acid and octadecanoic acid were detected as methylesters in this groundwater sample as shown in figure 5 by use of the mass trace m/z=74. At some localities, these acids reflect the activity of microorganisms in the aquifer (Martus, 2002). Concentrations are calculated using a response factor of 1 and yielded 4.46 µg/l for tetradecanoic acid, 8.39 µg/l for hexadecanoic acid and 7.3 µg/l for octadecanoic acid.

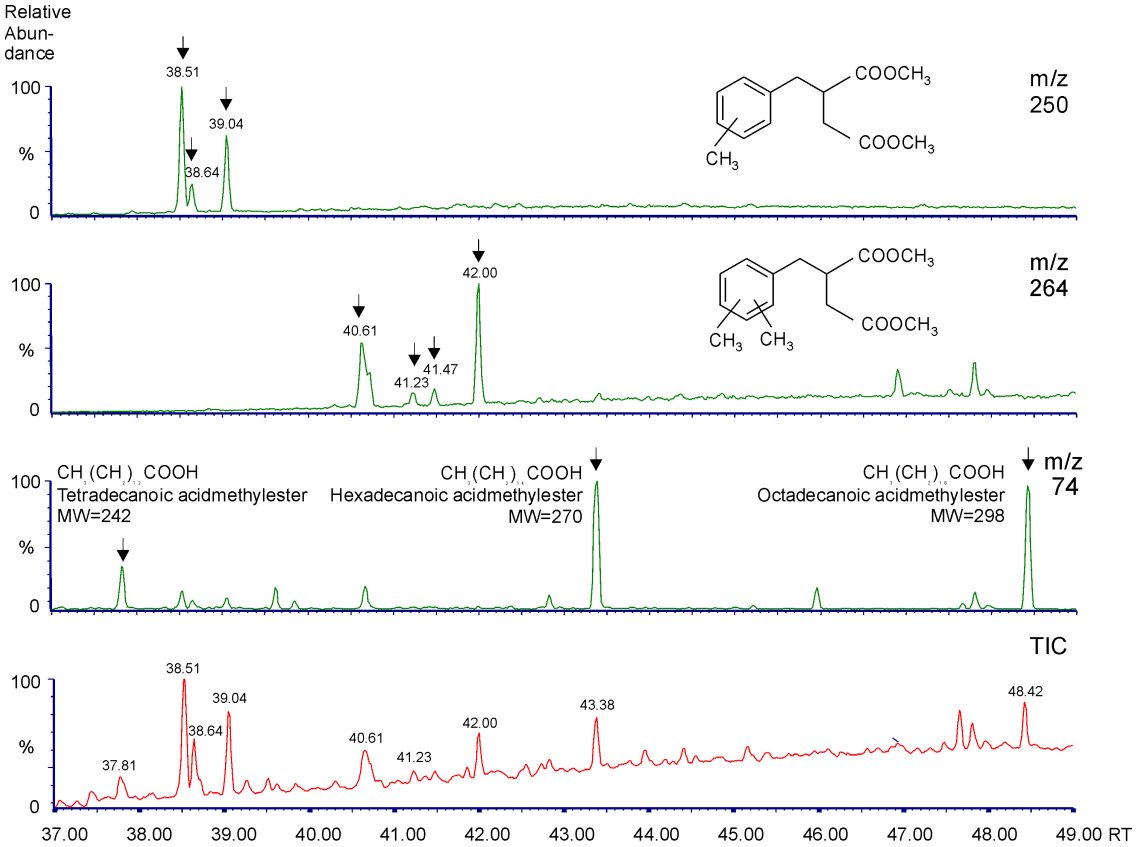

Figure 5: Distribution patterns of TMSH-derivatised aromatic acids in a TIC with the mass traces m/z=250 for methyl-BSA (as methylester), m/z=264 for C_2-BSA (as methylesters) and m/z=74 for the fatty acids tetradecanoic acid, hexadecanoic acid and octadecanoic acid (as methylesters) extracted from a BTEX contaminated groundwater sample. Retention time is given in minutes.

The analytical method was also applied for the identification and quantification of metabolites from PAH degradation. Authentic standard compounds for 2-naphthoic acid, tetrahydronaphthoic acid, methylnaphthoic acid and naphthyl-2-methylsuccinic acid are not commercially available. Thus, exact quantification for absolute contents for these compounds was not possible. In figure 6 are shown in the TIC and in the mass chromatograms for recording the compounds 2-naphthoic acid, isomers of tetrahydronaphthoic acid and methylnaphthoic acid. The aromatic acids 2-naphthoic acid and tetrahydronaphthoic acid were previously identified as metabolites of anaerobic degradation of naphthalene, 2-methylnaphthalene and 1,2,3,4-tetrahydronaphthalene (Meckenstock et al., 2000; Annweiler et al., 2002). The identification of 2-naphthoic acid and the isomers 1,2,3,4-tetrahydronaphthoic acid and 5,6,7,8-tetrahydronaphthoic acid was achieved by comparison with mass spectra published by Meckenstock et al. (2000); Gieg & Suflita (2002) and Safinowski (2005). Isomers of methylnaphthoic acid are detected in the mass trace m/z=200. The identification was done by adding 14 mass units to the mass of 2-naphthoic acid, corresponding to the additional methyl group.

Additionally, the metabolite naphthyl-2-methylsuccinic acid (see fig. 7) has been detected in a groundwater sample from the PAH contaminated site. Annweiler et al. (2000) have extracted and detected the metabolites naphthyl-2-methylsuccinic acid and naphthyl-2-methylenesuccinic acid. These compounds are generated by anaerobic degradation of 2-methylnaphthalene by a sulphate-reducing enrichment culture. The identification was done by comparison of the mass spectra published in Annweiler et al. (2000).

3.3.3 Conclusions

From monoaromatic BTEX hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) metabolites such as benzoic acid, benzylsuccinic acid, naphthoic acid and their homologs are generated in groundwater by biologically mediated degradation. The presence of the metabolites is used for the recognition for natural attenuation processes in contaminated groundwater.

The combination of solid-phase extraction based on octadecyl-bonded silica sorbent with gas chromatography coupled with mass spectrometry is a suitable method for the analysis of these metabolites. The decisive advantage of solid-phase extrac-

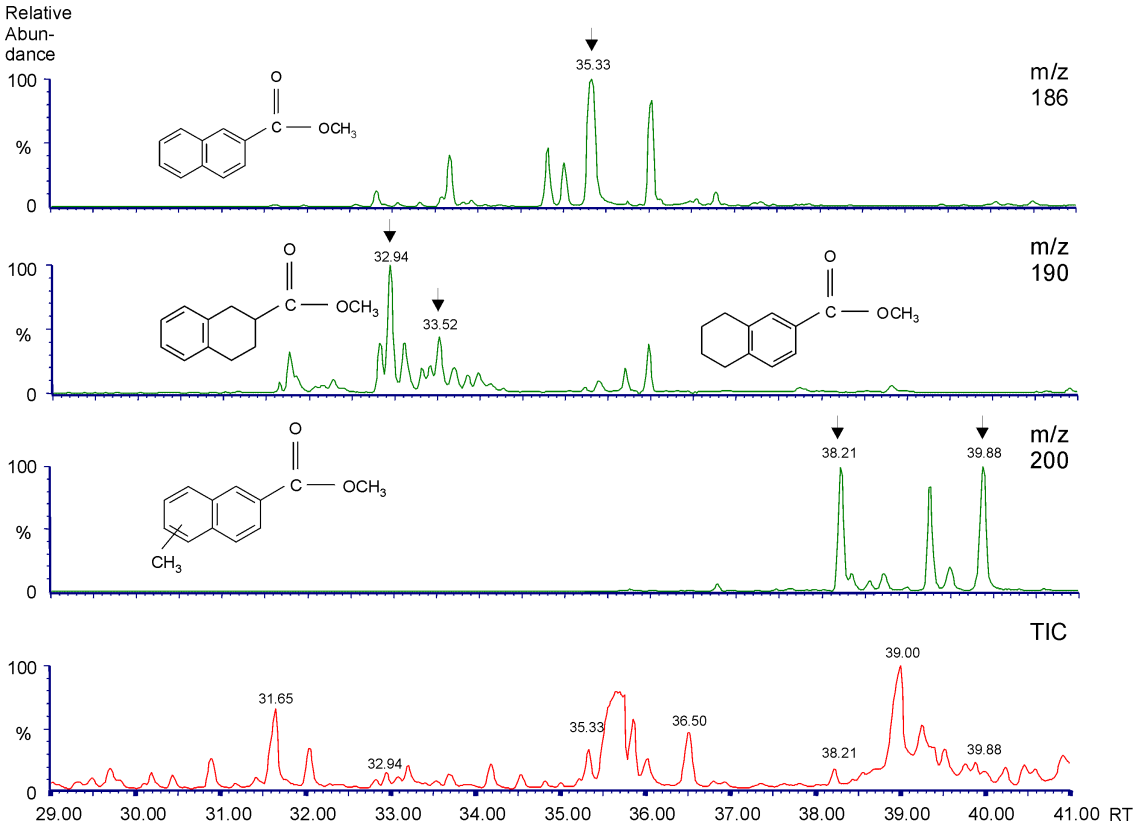

Figure 6: Distribution patterns of TMSH-derivatised aromatic acids in a TIC with the mass traces m/z=186 for 2-naphthoic acid, m/z=190 for the isomers of tetrahydronaphthoic acid and m/z=200 for the isomers of methylnaphthoic acid (as methylesters) extracted from a PAH contaminated groundwater sample. Retention time is given in minutes.

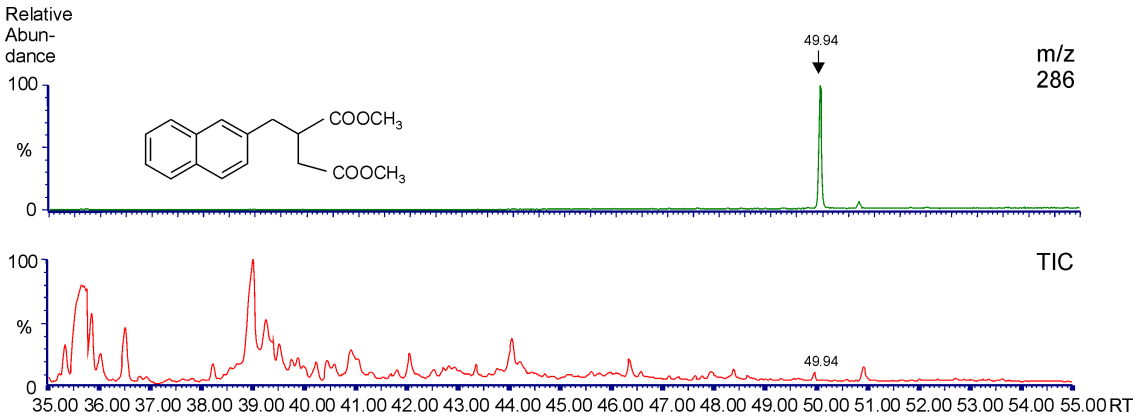

Figure 7: TMSH-derivatised aromatic acid naphthyl-2-methyl-succinic acid in a TIC with the mass trace m/z=286 (as methylester) extracted from a PAH contaminated groundwater sample. Retention time is given in minutes.

tion compared to liquid-liquid extraction is the reduction of huge amounts of solvents. Moreover, an enrichment of components with a factor of 1000 by extracting 1 ml eluate out of 1 l groundwater sample can be achieved and therefore the detection limits are low. Furthermore, the expenditure of time can be minimised by extraction of up to twenty samples at the same time.

4 Anaerobic biodegradation with a Sulphate- and Fe(III)-reduction and Methanogenesis overlap in a BTEX contaminated aquifer

Abstract

A long term study was carried out at the former military site Schäferhof-Süd, Nienburg/Weser (Germany) investigating the geochemical evolution of dominant terminal electron acceptor processes (TEAPs) in a contaminated aquifer. Groundwater contamination involving petroleum-hydrocarbons is a serious environmental problem. Monitored Natural Attenuation (MNA) is a passive remediation approach dealing with the degradation and dissipation of groundwater contaminants in situ. A large residual contamination involving benzene, toluene, ethylbenzene, xylene (BTEX) is present in sediment at the studied locality and groundwater samples indicate increasing BTEX concentrations in the groundwater flow direction. The depletion of oxygen, nitrate, and sulphate and the production of dissolved ferrous iron and methane in groundwater indicate biologically mediated processes in the plume evidently proving the occurrence of natural attenuation (NA). In the downstream sector, particulary high concentrations of up to 15 mg/l of CH_4 were detected. A large overlap of different redox zones has been observed. Furthermore, organic acids were identified as metabolic by-products of biodegradation. Benzoic acid, C_1-, C_2- and C_3-benzoic acid were determined in all contaminated wells with considerable concentrations.

4.1 Introduction

In recent years, the biodegradation process of petroleum hydrocarbons has been studied quite intensively with particular attention being paid to the subsurface and groundwater under various redox conditions (e.g. Christensen et al., 2000; Spormann & Widdel, 2000; Widdel & Rabus, 2001; Annweiler et al., 2002; Beller, 2002; Reusser & Field, 2002; Maurer & Rittmann, 2004; Schulze & Thiem, 2004; Kao et al., 2006; Farhadian et al., 2007). The biodegradation process is widely accepted today in the context of natural attenuation (NA) as an appropriate tool in dealing with subsurface contamination and controling the spread of contaminant plumes.

The crude oil constituents benzene, toluene, ethylbenzene, and the three xylene isomers (commonly referred to as BTEX) are the dominating groundwater contaminants originating from surface spill accidents. BTEX hydrocarbons are mainly introduced into the groundwater by oil production facilities and the contamination with gasoline and jet fuel (Wiedemeier et al., 1999; Cozzarelli & Baehr, 2003; Kao et al., 2006; Andreoni & Gianfreda, 2007).

An active cleanup procedure involving a petroleum hydrocarbons event is very expensive and the complete removal of the contaminants from the subsurface is almost impossible. As a result, a combination of active remediation focussing on the source of contamination and a monitoring of the residual contamination in the subsurface is often applied (Monitored Natural Attenuation; MNA) (e.g. Wiedemeier et al., 1999; Martus, 2002; Hinspeter & Püttmann, 2003; Farhadian et al., 2007).

Measurable changes of biologically sensitive geochemical parameters in the groundwater taken from a contaminated area can indicate intrinsic processes of biodegradation requiring hydrological, geochemical and microbiological conditions advantageous for the transformation and metabolism of contaminants into less harmful products. Intrinsic bioremediation relies on naturally occurring microbial processes for pollutant degradation and the containment in sediment and groundwater without the added delivery of nutrients, electron acceptors or other stimulants.

Naturally occurring electron acceptors commonly consumed in the microbial metabolism of organic contaminants include dissolved oxygen, nitrate, ferric iron, sulphate and carbon dioxide. Due to the increased organic loading of contaminated aquifers, oxygen is usually rapidly depleted and biodegradation continues under anaerobic conditions dependent on the utilisation of alternate soluble and insolu-

ble electron acceptors. The effectiveness of the hydrocarbon degradation process is controlled by the availability of alternate electron acceptors. The process can be monitored by measuring the depletion of the electron acceptors in the contaminated area and the accumulation of dissolved metabolites such as organic acids, methane and carbon dioxide and comparing this with a non-contaminated upstream groundwater (Weiner & Lovley, 1998; Heider et al., 1999; Beller, 2000; Spormann & Widdel, 2000; Phelps & Young, 2001; Johnson et al., 2003; Martus & Püttmann, 2003; Aitken et al., 2004; Chakraborty & Coates, 2004; Gödeke et al., 2006; Kao et al., 2006; Gaab et al., 2007; Morasch et al., 2007).

Based on the concept that more energy-yielding electron acceptors are consumed before less energy-yielding ones, terminal electron accepting processes (TEAPs) have often been approximated as occurring sequentially (Stumm & Morgan, 1996). Thereby, redox zones with limited overlap zones can be observed. For instance, sequential electron acceptor utilisation can theoretically produce methane at the source of contamination and Fe(II) further down gradient, hence resulting in a limited overlap of Fe(II) and methane. However, field observations have shown that contaminant plumes sometimes display far more extensive overlap zones (Gieg et al., 1999; Cozzarelli et al., 2000; Schreiber et al., 2004; Bianchin et al., 2006; Roychoudhury & Merrett, 2006). Previous studies have used the distribution of redox-sensitive constituents in identifying the governing redox processes, discussing the difficulties in defining distinct redox zones in hydrocarbon-contaminated aquifers based soley on geochemical indicators. The major difficulties arise with mixed signals (Lovley et al., 1994a; Bjerg et al., 1995; Chapelle et al., 1995, 1996; Heidrich et al., 2004). Temporal and spatial variations of the dominant TEAPs can shift as hydrological and geochemical conditions change in an anoxic aquifer, resulting in different rates of degradation of the organic contaminants. Beeman & Bleckmann (2002) report on the successful decontamination of benzene from groundwater with the sequential change of aerobic and anaerobic conditions. The dominant anaerobic biodegradation processes applied in the Beeman & Bleckmann (2002) study were active under sulphate-reducing and methanogenic conditions. Investigations in a petroleum-contaminated aquifer (Weiner & Lovley, 1998) and experiments with BTEX-contaminated groundwater resulting from a gasoline spill (Reinhard et al., 2005) have shown that in the absence of any other electron acceptors, benzene has

been converted to CH_4 and CO_2 with no lag phase. It has thereby been observed, that the presence of TEAs (oxygen, nitrate, iron, sulphate) is not necessary for natural attenuation to occur.

In this particular study, intrinsic bioremediation has been shown to occur at the former military site Schäferhof-Süd with identifying residual contamination, mainly BTEX in the subsurface. The BTEX concentration in groundwater increases with the flow direction due to the superimposition of various BTEX sources. The aim of this study was to identify the distribution of redox-sensitive groundwater constituents in the aquifer, identify the governing redox environments in the plume and thereby provide evidence of biologically mediated BTEX degradation.

In investigating the BTEX contamination, the TEAPs and metabolites of BTEX degradation in groundwater, the five monitoring wells (MP1 - MP5) were sampled. Additionally, vertical sediment profiles from the area of the former storehouse of fuel were investigated with respect to spreading and concentration of BTEX in the subsurface.

4.2 Sampling and analytical methods

4.2.1 Field sampling procedures

Groundwater samples were collected at the study site along the groundwater flow-path transect (fig. 1 and 2 in chap. 2, p. 21, 22) over a period of three years between January 2002 and September 2004. Samples were taken in regular intervals of three months from the double level monitoring wells analysing BTEX, oxygen (O_2), nitrate (NO_3^-), sulphate (SO_4^{2-}) and ferrous iron (Fe^{2+}). The sampling procedure focussed on measuring the parameters pH, temperature, electric conductivity and oxidation-reduction potential. Methane samples were collected in 2002 and 2003 twice a year (June and December) and in 2004 three times per year (March, June and September). Metabolites altogether were sampled at five occasions (2002 and 2003 twice a year each in June and December and once in June in 2004).

The groundwater samples were collected with a submersible pump (GRUNDFOS, type MP1). Prior to the water sampling phase, a minimum of three volumes of water were removed from the wells until the parameters temperature, pH, conductivity and content of oxygen in the groundwater finally stabilised. 1 l of unfiltered

groundwater was collected for each sample and stored in sterilised dark glass bottles in order to determine contaminants, electron acceptors NO_3^-, SO_4^{2-} and products of biodegradation Fe^{2+} and metabolites. 20 ml of groundwater was collected and stored in headspace glasses and capped air-tight facilitating the analysis of methane. All samples were stored cool at approximately 4℃ during transport to the laboratory.

Sediment sampling at the area of the fuel storehouse was completed in October 2004. These drillings were performed as double face drillings. Samples were obtained by sinking two opposite drillings (liner, DN 100) per drilling area (diameter 1 m) to a depth up to 8 m. These double-face drillings were performed to test the homogeneity of the subsurface material. Every meter over the whole range of a drilling was homogenised to one sample. A 2 g sample equivalent was collected and stored in headspace glasses and closed gas-tight in order to determine the BTEX content. Samples arrived at the laboratory and were analysed within 24 hours. Refer to figure 1 in chapter 2 for the location of the drilling areas B3 and B5. All groundwater and sediment samples were taken by Uwe Drewes, Alphacon GmbH, Ganderkesee.

4.2.2 Analytical techniques

Temperature, electric conductivity, pH, Eh and O_2 were determined in flow cells connected directly to a pump discharge using electrodes. A portion of groundwater samples were outsourced to the commercial laboratory of Alphacon GmbH, Ganderkesee for the analysis of BTEX, NO_3^-, SO_4^{2-} and Fe^{2+}. The analysis of CH_4 were done at the laboratory of the Wessling Holding GmbH & Co KG, Altenberge. The BTEX analysis in groundwater and sediment samples followed the DIN 38407-F9 (equivalent with EPA Method 8020).

In compliance with DIN 38407-F9, 2 ml of each groundwater sample was filled in headspace-vials, capped gas-tight and heated for one hour to a temperature of 80℃. The gas mixture from the headspace above the groundwater sample was then analysed by gas chromatography/mass spectrometry (GC/MS) using a HP 5890 GC coupled with a MSD HP 5971. Similarly, 2 g of each sediment sample and 1 ml deionised water were filled in headspace vials and capped gas-tight. The sediment samples were heated for one hour to a temperature of 80℃ and analysed. The BTEX analysis was carried out within 24 hrs after sampling and the method detection limit

(MDL) recorded 1 µg/l for groundwater and 100 µg/kg for sediment.

Nitrate and sulphate concentrations in groundwater were determined using photometric methods. The MDL for both nitrate and sulphate concentrations equalled 1 mg/l. The analysis of Fe^{2+} was performed using atomic absorption spectroscopy (AAS) (MDL 0.02 mg/l). Methane was analysed using headspace techniques by gas chromatography coupled with a flame ionisation detector. The MDL was 10 µg/l.

Metabolites in groundwater were analysed using a solid-phase extraction technique coupled with a GC/MS, described in detail in chapter 3. The MDL was 2 µg/l.

4.3 Results

4.3.1 Contaminant distribution

The subsurface of the study area contains a large residual contamination of petroleum derived hydrocarbons dominated by benzene, toluene, ethylbenzene and xylene (BTEX) (Hettwer et al., 2006; Hettwer, 2006). Investigations of the BTEX contamination of sediment samples taken from profiles in the area of the former storehouse at the drilling points B3 and B5 are shown in figure 8. In the unsaturated zone a maximum value of 17 mg/kg BTEX (drilling point B3/3) is measured, which increases up to 120 mg/kg (drilling point B3/3) in the capillary fringe. In the saturated zone at drilling point B3/4, a maximum value of 6.7 mg/kg BTEX is detected. In figure 8, the minimum and maximum of groundwater level for the period of monitoring is marked. The highest BTEX contamination is detected at a depth range between 5 and 6 meters. The zone of elevated hydrocarbon concentration within the sediments can be correlated with the zone of the lowest observed groundwater table elevation.

Table 5 presents mean analytical results for concentrations of benzene, toluene, ethylbenzene, m+p-xylene, o-xylene and the sum of BTEX in groundwater. Groundwater samples collected at the well MP1, located upstream from the source of the contamination were free of contaminantion over the entire monitoring period and were thus used as a reference for comparison with other observation wells. In the upper screened wells in flow direction, a distinct increase of BTEX concentrations from 532 µg/l in MP2-f up to 3300 µg/l in MP5-f is observed (mean values; see

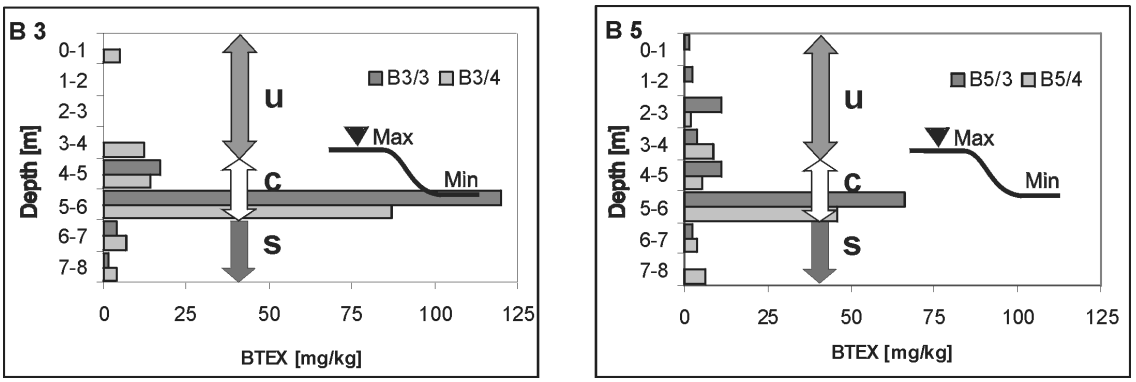

Figure 8: Depth profiles of sediments at drilling locations B3 and B5 (see fig. 1) at the former fuel storehouse indicating BTEX-concentrations detected in the sediments of every meter of the double face drillings. The varying groundwater level is also shown in the observation well MP2 (upper screened well) during the sampling period from Jan. 2004 to Sep. 2004. u=unsaturated zone, c=capillary fringe, s=saturated zone (modified after (Gaab et al., 2007)).

tab. 5). For the lower screened wells, with the exception of MP5, almost no BTEX could be detected. In well MP5, very high concentrations of BTEX have been measured consistently. 2850 µg/l median BTEX concentration values were recorded in the upper well MP5 and 3100 µg/l in the lower well (fig. 9). The upper well MP5 shows the highest observed BTEX concentrations 6.900 µg/l whilst 5300 µg/l was recorded at the lower section MP5. The observed distribution of BTEX concentrations indicates that the available wells cover only a part of the plume originating from the former fuel storehouse. A further source of BTEX must be present downstream and in the area of the monitoring well MP5. This is supported by the observed increase of BTEX concentrations in the flow direction (fig. 9). In the groundwater flow direction, from the observation well MP2 to MP3, an increase of easily biodegradable benzene (from 12 µg/l up to 50 µg/l) and toluene (112 µg/l up to 179 µg/l) concentrations is observed. Also, the concentrations of the more persistent compounds ethylbenzene and o- ,m- & p-xylene increase in the direction of groundwater flow. In the area of the observation wells MP4 and MP5, multiple additional sources of BTEX can be assumed in the subsurface because of the tremendous rise of the concentrations of toluene, ethylbenzene and the isomers of xylene. At the beginning of this study, the source of contamination was only known to be located at the former fuel storehouse.

Table 5: Analytical results obtained from sampling of groundwater at the observation wells MP1-MP5 from January 2001-September 2004. Note that high variations involving parameters are mostly due to seasonal variations. Values given as mean ± SD.

Location	MP1 (upstream)		MP2 (source of cont.)		MP3 (downstream)		MP4 (downstream)		MP5 (downstream)	
	MP1-f	MP1-t	MP2-f	MP2-t	MP3-f	MP3-t	MP4-f	MP4-t	MP5-f	MP5-t
Distance to MP1 (m)	0		25		50		50		100	
Benzene (µg/l)	**0**±0	**0**±0	**12**±7	**0**±0	**50**±25	**1**±1	**8**±3	**0**±0	**57**±34	**159**±53
Toluene (µg/l)	**0**±0	**0**±0	**5**±3	**0**±0	**11**±6	**0**±1	**261**±111	**0**±1	**681**±403	**443**±136
Ethylbenzene (µg/l)	**0**±0	**0**±0	**112**±85	**0**±1	**179**±106	**1**±2	**508**±304	**2**±2	**498**±276	**605**±252
m+p-Xylene (µg/l)	**0**±0	**0**±0	**390**±269	**1**±2	**579**±330	**3**±4	**1793**±1058	**5**±4	**1618**±1002	**1531**±741
o-Xylene	**0**±0	**0**±0	**12**±7	**0**±1	**14**±7	**1**±1	**596**±354	**1**±3	**454**±228	**425**±195
Sum of BTEX (µg/l)	**0**±0	**0**±0	**532**±363	**2**±3	**834**±468	**6**±8	**3161**±1772	**8**±8	**3300**±1814	**3178**±1342
pH	**6.6**±0.2	**5.7**±0.3	**6.4**±0.2	**5.9**±0.2	**6.3**±0.2	**6.3**±0.3	**6.4**±0.2	**6.2**±0.2	**6.6**±0.2	**6.6**±0.2
Eh (mV)	**450**±40	**455**±39	**94**±46	**386**±65	**134**±34	**296**±61	**106**±32	**299**±56	**111**±23	**84**±13
electric cond. (µS/cm)	**256**±41	**332**±33	**394**±114	**319**±53	**398**±61	**268**±28	**411**±73	**206**±30	**518**±136	**504**±49
diss. Oxygen (mg/l)	**5.2**±1.7	**0.6**±1.3	**0.7**±1.1	**1.1**±1.4	**1.2**±1.5	**1.5**±1.5	**0.4**±0.6	**3.5**±2.0	**0.5**±0.5	**0.4**±0.6
Nitrate (mg/l)	**45**±12	**68**±11	**4.5**±5.1	**63**±11	**4.7**±4.3	**38**±16	**1.6**±3.8	**27**±8.9	**0.9**±1.4	**0.1**±0.3
Sulphate (mg/l)	**28**±3.7	**38**±3.8	**25**±14	**36**±7.1	**25**±9.8	**29**±5.4	**9.9**±6.4	**26**±6.7	**19**±9.4	**1.9**±1.8
Ferrous iron (mg/l)	**0.15**±0.29	**0.09**±0.15	**40**±18	**0.04**±0.03	**33**±11	**0.06**±0.07	**43**±10	**0.19**±0.17	**30**±19	**74**±16
Methane (µg/l)	**0**±0	**0**±0	**1227**±285	**0**±0	**5543**±999	**35**±53	**8571**±2554	**87**±197	**2614**±1354	**9300**±2955
Benzoic acid (µg/l)	**0.7**±0.9	**5.0**±6.0	**3.8**±3.6	**1.8**±1.5	**2.6**±2.5	**2.3**±2.0	**4.0**±5.2	**1.2**±1.4	**1.5**±1.9	**0.7**±1.2
$C_1$1-benzoic acid (µg/l)	**0**±0	**0.1**±0.3	**10.3**±12.2	**0.2**±0.3	**6.5**±7.3	**0.3**±0.4	**30**±17	**0.2**±0.2	**7.7**±6.4	**14**±13
C_2-benzoic acid [*1*2] (µg/l)	**0**±0	**0**±0	**142**±16	**0.2**±0.2	**83**±34	**0.3**±0.4	**115**±44	**0.7**±0.9	**20**±13	**97**±30
C_3-benzoic acid [*1*3] (µg/l)	**0**±0	**0**±0	**91**±118	**0.1**±0.2	**46**±32	**0.3**±0.5	**61**±21	**0.1**±0.1	**16**±5.3	**69**±11

[*1] sum of isomers

[*2] dimethyl benzoic acid or ethyl benzoic acid

[*3] trimethyl benzoic acid or methyethyl benzoic acid

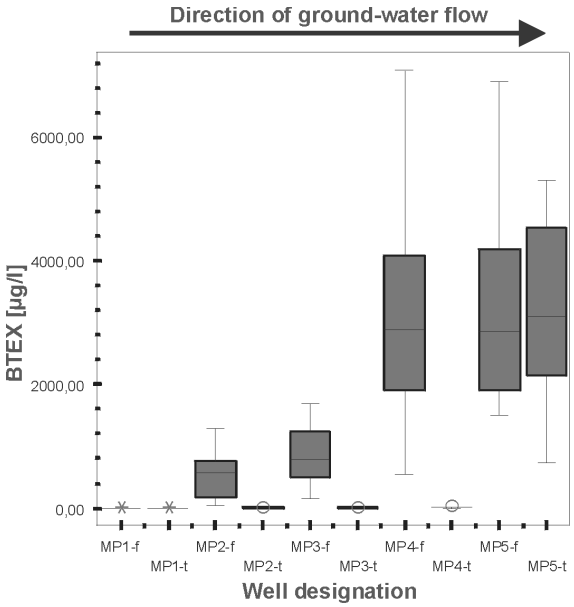

Figure 9: Boxplots of BTEX-concentrations in groundwater (n=12). The monitoring wells MP1 - MP5 are double level wells with one upper section (f) and one lower section (t). The boxplots show the variation limits of concentrations at individual points in the groundwater flow direction during the entire sampling campaign from January 2002 to September 2004. There is no evidence for temporal variations. (The bottom and top of the box are always the 25th and 75th percentile, and the band near the middle of the box is always the 50th percentile (the median). The whiskers represent the lowest datum still within 1.5 interquartile range (IQR) of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile; o = outlier; * = extreme value)
An increase of the contaminants BTEX is observed in the upper section in the direction of groundwater flow. MP5 also demonstrates contamination at the lower section.

4.3.2 DO and Eh measurements in groundwater flow direction

The parameters dissolved oxygen (DO) and Eh indicate the occurrence of anaerobic conditions within the source area and downstream area. In MP1-f (upstream from the source), the dissolved oxygen (DO) in groundwater amounts to 5.2 mg/l (mean value). In nearly all contaminated observation wells, DO is lower than 0.52 mg/l, with the exception of the measurements in June and September of 2003 at the observation well MP4-t and in December 2003 at the well MP3-f. 5.9 and 6.3 mg/l were observed at MP4-t respectively and a content of 4.9 mg/l at MP3-f.

Relative to the reference well MP1-f with a mean value of 450 mV in groundwater, the Eh decreases to 84 mV taken from the downstream locations due to the microbiological activity and the consumption of nutrients. Values of 450 mV Eh

in groundwater are very high (Appello & Postma, 1996) but not unusual. Kao & Wang (2001) have also detected background values in this range. The decreasing Eh-values in the contaminant plume reflect the change from oxidising to reducing conditions. The pH remains constant in the contaminant plume (tab. 5).

4.3.3 Degradation of BTEX indicated by TEAPs

The concentration of the redox components NO_3^-, SO_4^{2-}, Fe^{2+} and CH_4 along the transect is shown in figure 10 and the analytical results obtained from sampling of the groundwater are presented in table 5. At the observation well MP1-f, mean nitrate concentrations of 45 mg/l were detected. In the majority of the sampled wells in the contaminated section of the aquifer, the nitrate concentrations tend to move towards zero in the groundwater flow direction. After a decrease of DO, which is observed in nearly all contaminated groundwater samples, denitrification starts if nitrate is present in sufficient concentrations (Wiedemeier et al., 1999). In general, low levels of nitrate were detected with mean values of 0.1-1.6 mg/l at the observation wells MP4-f (upper section) and MP5 (upper and lower section), whereas at the other two contaminated wells small amounts were detected (4.5 and 4.7 mg/l).

The reduction of sulphate in the groundwater does not follow regular spatial and temporal trends. In the areas of the former fuel storehouse and downstream at MP3-f, sulphate concentrations were recorded with 25 mg/l showing minimal reduction observations compared with 28 mg/l (see tab. 5 and fig. 10) at the upstream well. Increased sulphate concentrations were detected temporarily in well MP2-f. In contrast, the concentrations dropped to 1.9-9.9 mg/l (see tab. 5) in the wells MP4-f, MP5-f and MP5-t. A decrease of sulphate in the groundwater flow direction from 38 mg/l in MP1-t up to 26 mg/l in MP4-t to 1.9 mg/l in MP5-t has likewise been observed in the lower screened sections of the wells (see tab. 5 and fig. 10).

Low Fe(II) concentrations were detected in the background water samples (0.15 mg/l) and in the lower sections within the contaminated plume (0.04-0.19 mg/l) with the exception of MP5-t. Within the contaminated plume however, 30 mg/l - 43 mg/l of dissolved Fe(II) was measured at the BTEX-contaminated upper sections of the observation wells and 74 mg/l at the lower section of MP5 (tab. 5 and fig. 10). Methane is observed in all contaminated wells. In the area of the fuel

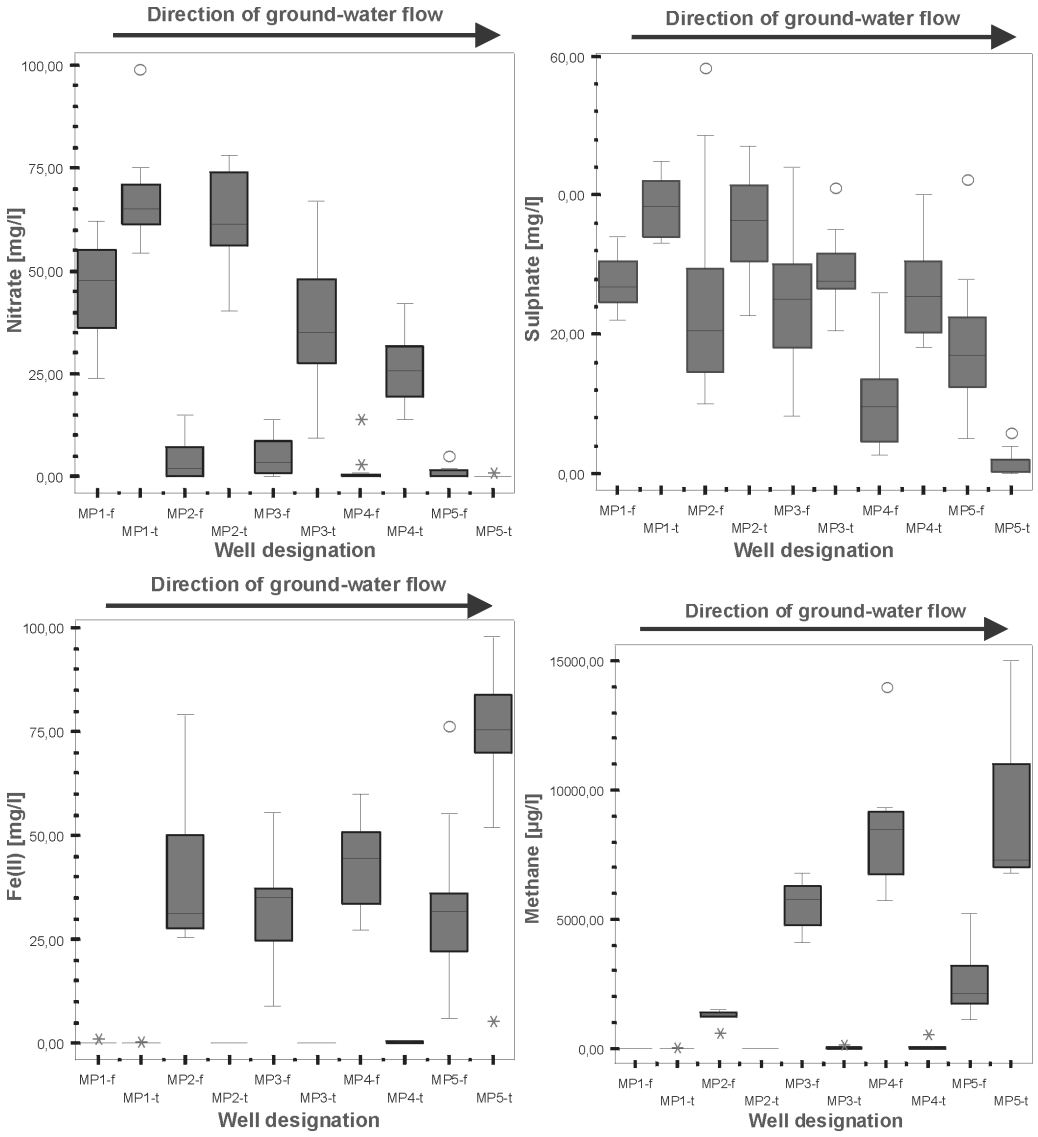

Figure 10: Concentration of redox components shown as boxplots: nitrate (n=12), sulphate (n=12), iron(II) (n=12) and methane (n=7) in groundwater (Jan.2002-Sep.2004). For location of sampling sites, see figures 1 and 2 in chapter 2.

storehouse, concentrations of 1227 µg/l were detected (tab. 5 and fig. 10). In the downstream area, elevated concentrations up to a maximum of 15 mg/l were observed (well MP5-t). In the uncontaminated wells MP3-t and MP4-t, small amounts of methane (35 µg/l and 87 µg/l) were also detected. These analytical data indicate the simultaneous occurrence of nitrate, sulphate and iron reduction as well as methanogenese within the contaminated aquifer.

4.3.4 Metabolites (organic acids)

If microbiological processes are operative in a contaminated aquifer, one might expect substantial changes in the hydrogeochemistry of electron acceptors and also in the composition and metabolism of the contaminants relative to a reference area. Due to the low solubility of oxygen in groundwater, hydrocarbon contaminated aquifers usually become anaerobic and the major fraction of contaminants degrade in the anaerobic zones of the plume (Wisotzky & Eckert, 1997; Beller, 2000; Spormann & Widdel, 2000; Phelps & Young, 2001; Chakraborty & Coates, 2004). During biologically mediated BTEX degradation under anaerobic conditions, organic acids are generated as intermediates supported by bacteria. The detection of these metabolites, which are compounds such as benzoic acid (BA) and the methylated homologs, is an established approach to confirm the biodegradation of hydrocarbons in a contaminated aquifer (e.g. Beller, 1995, 2002; Elshahed et al., 2001).

In the present study, an increase in metabolic products of monoaromatic hydrocarbons has been found in the groundwater in the contaminated area downstream from the background area (MP1). However, BA was also observed in MP1 and in the lower, uncontaminated parts of the aquifer in low concentrations (up to 5.0 µg/l) indicating that this compound is not only a specific toluene degradation product but has to be attributed at least partly to natural sources. In all contaminated wells, organic acids are detected in distinctive concentrations (see tab. 5). Particularly at MP2-f, the concentrations of C_2- and C_3-benzoic acid with mean values of 142 µg/l and 91 µg/l increased.

As shown in table 5, maximum concentrations of BTEX do not correlate with increased concentrations of metabolites. At the well MP5-f, mean BTEX concentrations of 3300 µg/l were detected yet only a mean value of 45 µg/l metabolites (sum of mean values from the metabolites: BA, C_1-,C_2- and C_3-BA) was observed. At the observation well MP4-f, high BTEX concentrations of 3161 µg/l were detected,

which was accompanied by elevated metabolic concentrations (210 µg/l; sum of mean values from BA, C_1-,C_2- and C_3-BA). The variations of the metabolites C_2- and C_3-benzoic acid in the monitoring time-span correlate with the seasonal variations of the groundwater level. This is shown exemplarily for MP2-f in figure 11. In the summer of 2003, the groundwater table increased to 24.6 m ASL and decreased in the following winter to a level of 23.5 m ASL. Parallel to this progressive curve, metylated BAs concentrations increase and decrease. The BA concentrations however, do not follow this pattern.

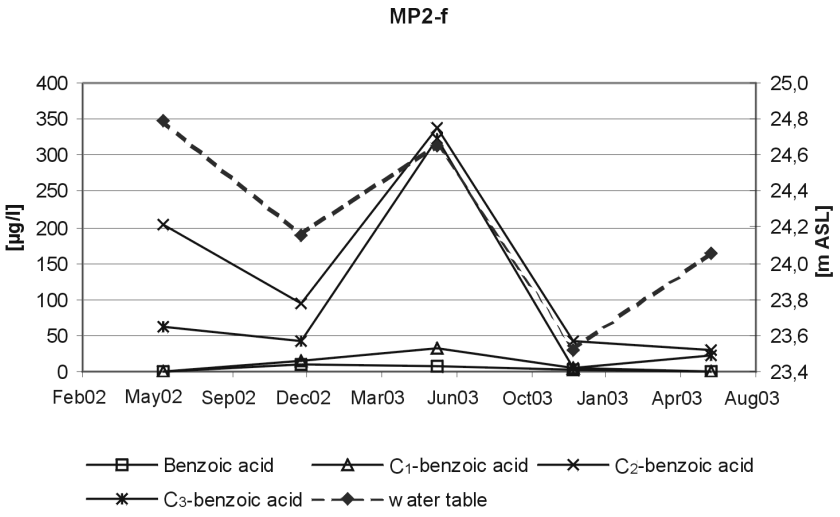

Figure 11: Correlation of variations in concentrations of metabolites C_1-; C_2- and C_3-benzoic acids with seasonal variations of groundwater table at the observation well MP2-f.

4.4 Discussion

In the vertical sediment profiles B3 and B5 at the former military site Schäferhof-Süd, BTEX concentrations show that the contamination disperses inhomogenously. In figure 8, a rapid increase of BTEX concentrations is shown at a depth of 5 to 6 meters. This depth range correlates with the capillary fringe and the lowest observed groundwater table elevation. A significant influence of groundwater table fluctuation on the vertical distribution of hydrocarbons in sediment has also been found for other contaminated sites (Cheol-Hyo et al., 2001; Klonowski et al., 2008).

BTEX are badly soluble in water and mostly present as a separate liquid oil phase in the subsurface (see tab. 1). After spill accidents, hydrocarbons migrate to the capillary fringe and disperse in the unsaturated zone and also into the sector of groundwater level variations. BTEX leaches from the oil phase in the sediment at the capillary fringe into the groundwater due to the influence of seepage water and due to groundwater level variations. Subsequent to the gasoline leakage at the investigation site, anaerobic conditions have developed within the contaminated source and downstream area. O_2-values are low in nearly all contaminated observation wells. Thus, anaerobic biodegradation can be expected to be the dominant biodegradation process within the contaminated aquifer.

The spatial distribution of the major TEAs suggests the occurrence of nitrate, iron and sulphate reduction and methanogenese within the contaminant plume (fig. 10). The temporarily elevated DO concentrations at the observation wells MP3-f and MP4-t are in conflict with the TEAs measurements. Nevertheless, residual concentrations of oxygen in the groundwater is present. Likewise, NO_3^-, SO_4^{2-}, Fe^{3+} reduction and methanogenese occurs (tab. 5). In homogeneous environments, the reduction of electron acceptors in the sequence O_2, NO_3^-, Fe^{3+}, SO_4^{2-} to CO_2 is largely true. Furthermore, it has been shown in the literature that Fe(III) reduction and SO_4^{2-}-reduction exclude each other, SO_4^{2-}-reduction and methanogenese also (Wiedemeier et al., 1999). This is based on the principle, that the preceding electron acceptor of the respective referred sequence can be toxic to microorganisms capable of using its following electron acceptor.

In natural environments however, different anaerobic redox processes occur simultaneously on a wide range of temporal and spatial scales due to subsurface heterogeneities (Gieg et al., 1999; Cozzarelli et al., 2000; Schreiber et al., 2004; Bianchin et al., 2006; Roychoudhury & Merrett, 2006). Due to these heterogeneities, an overlap of redox processes develops. The heterogeneities are on the one hand due to various particle sizes with the occurrence of organic matter in sediment and on the other hand due to the heterogeneous bioavailability of TEAs in the subsurface. This could be an explanation for the existence of oxygen together with increased concentrations of Fe^{2+} and CH_4 in groundwater taken from one particular well (e.g. MP3-f, tab. 5). The increased concentrations of sulphate at the observation well MP2-f could be explained by variations in the source-material. The first two me-

ters of subsurface material consists of filling material containing building rubble, well known for its emission of sulphate into seepage water (Hornbruch et al., 2007). Due to this, intensive sulphate reduction in the groundwater is possible, but is not evident by the measurements of sulphate concentrations in groundwater.

In the contaminated area, a significant increase of ferrous iron concentrations has been detected (fig. 10). This can be explained by variations in the contamination source or by means of a reduction of solid Fe(III) minerals in the sediment. The most probable explanation is iron reduction, a microbial mediated process which generates ferrous iron soluble in water. Iron-reducing cultures are able to oxidise BTEX compounds in contaminated sediments (Anderson & Lovley, 1999; Jahn et al., 2005). At the observation wells MP4-f and MP5-t, a parallel reduction of sulphate and Fe(III) has been observed. Similar observations have been obtained by Roychoudhury & Merrett (2006) at the Cape Flats Aquifer spill site in South Africa. The concomitant reduction of nitrate, iron, manganese and sulphate was observed here to.

Figure 12 shows that no distinct redox zones have formed at the investigation site Schäferhof–Süd. Overlap zones have however formed due to temporal and spatial variations involving hydrological and geochemical conditions. Zones with different redox status have likewise formed. Reduced nitrate and sulphate concentrations and increased ferrous iron and methane concentrations have been observed in all contaminated wells. When sulphate reduction and iron reduction take place simultaneously, Fe^{2+} and hydrogen sulphide are partly removed from the solution by rapid precipitation of immobile iron sulphide minerals (Ulrich et al., 2003) as Fe^{2+} is highly reactive. The coexistence of methanogenese and sulphate reducers have also been observed by Beeman & Suflita (1990); Beeman & Bleckmann (2002) and Heidrich et al. (2004). During the sampling of groundwater from the observation wells, it must be pointed out that no contamination of water from other microenvironments with different redox status could have occurred as data was collected consequently over an observation period of three years.

Methanogenesis is then possible when relatively small amounts of free energy is produced by degradation processes and not by means of the favoured thermodynamical reaction. Methanogenesis takes place in environments that lack other electron acceptors or takes place after other electron acceptors are depleted (Wiedemeier

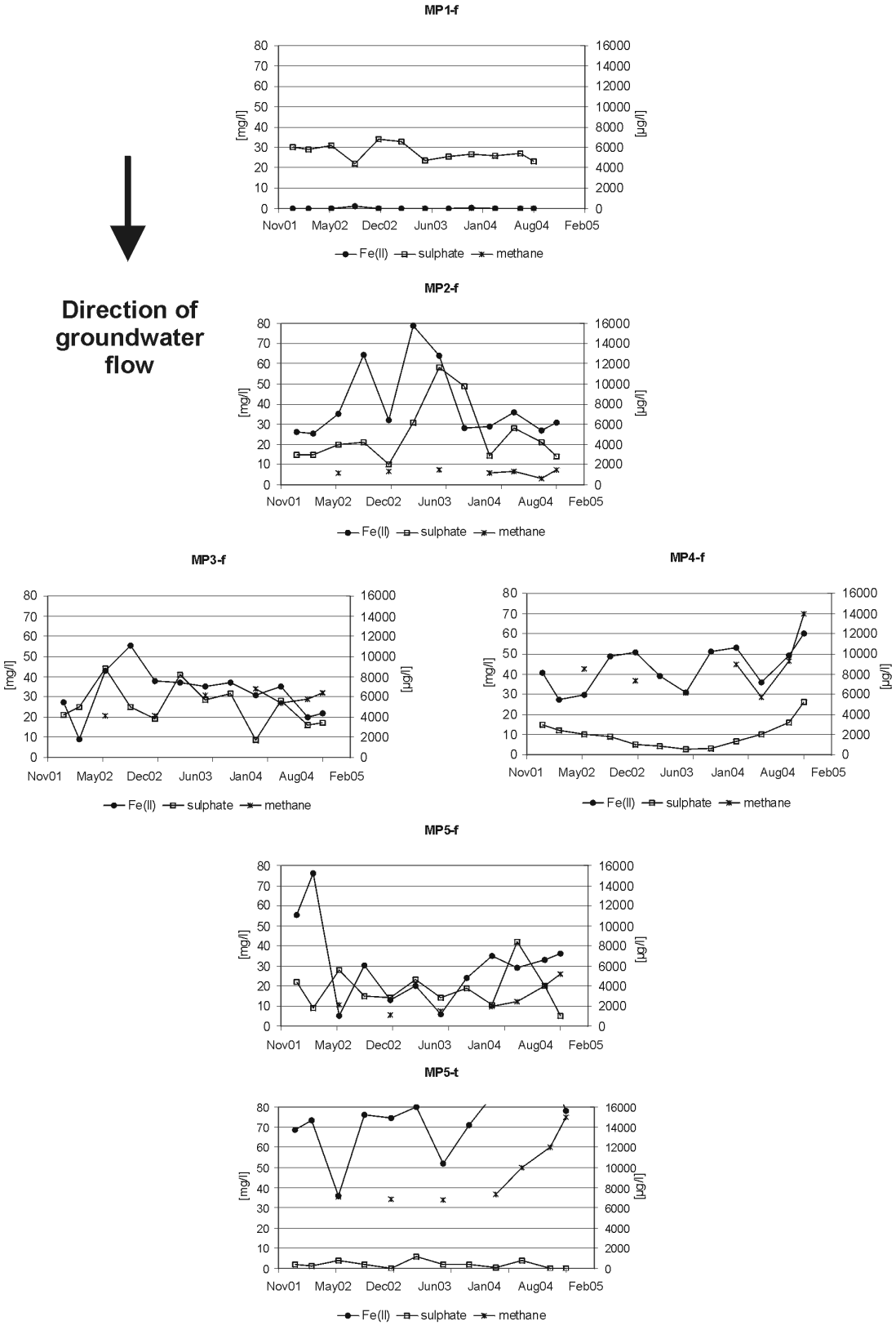

Figure 12: Ferrous iron, sulphate and methane concentrations in the contaminated wells at the study site.

et al., 1999). Several field studies have also shown an extensive Fe(II) and methane overlap (Cozzarelli et al., 1994; Jakobsen & Postma, 1999; Heidrich et al., 2004; Schreiber et al., 2004; Bianchin et al., 2006). The high Fe(II) and methane concentrations in the groundwater samples of Schäferhof–Süd reveal that Fe reduction and methanogenesis indicate dominant biodegradation patterns within the plume.

The occurrence of metabolic products of monoaromatic hydrocarbons in the contaminated observation wells provide evidence for BTEX degradation under anaerobic conditions. The metabolites benzoic acid (BA) and their methylated homologs are proposed as being indicators of BTEX degradation processes under anaerobic conditions (Beller, 1995, 2002; Elshahed et al., 2001). BA is an intermediate of the toluene degradation (Biegert et al., 1996). It is not however, an ideal biogeochemical indicator because of its common use in commercial products and its intermediate position during the anaerobic metabolism of other various aromatic compounds (Beller, 1995). Alkylated forms of BA are better indicators in identifying biodegradation of petroleum derived pollutants such as kerosene or gasoline in groundwater. Aromatic acids are formed by the oxidation of the methyl group. Biegert et al. (1996) identified benzylsuccinates which are generated in an intermediate step in the degradation of toluene under anaerobic conditions. Methylbenzylsuccinate is an intermediate of the C_2-benzene to methylbenzoic acid degradation path (Morasch & Meckenstock, 2005). Similarly, C_3- and C_4-benzenes represent the parent hydrocarbons for the C_2- and C_3-benzoic acids respectively (Beller, 2000).

Increased concentrations of C_1-, C_2- and C_3-benzoic acid are detected in the upper sections of the contaminated observation wells at the study site (see tab. 5). Figure 11 shows a correlation between the metabolites C_2- and C_3-BA variations and the seasonal groundwater variations at MP2-f. It is assumed, that due to dilution processes of BTEX from sediment with seepage water, induced by an increase of the groundwater table in the summer of 2003, this posed as ultimate stimulant in the degradation of contaminants. Furthermore, an increase of the electron acceptor sulphate is observed in June 2003 at MP2-f. Increasing amounts of nutrients and electron acceptors apparently support the biodegradation processes in groundwater as reflected by increasing amounts of metabolites from alkylated benzenes in the groundwater.

4.5 Conclusions

At the study site Schäferhof-Süd, an unexpected spatial distribution of contamination is observed in the subsurface. The former fuel storehouse was anticipated to be the dominant point source of contamination whereby downstream, decreasing concentrations of hydrocarbons were expected. However, the BTEX concentrations increase with the groundwater flow direction due to additional sources of BTEX located in the down gradient area. The determination of degradation rates revealed however to be quite impossible. A variety of hydrochemical and biochemical approaches were required to test the hypothesis that intrinsic bioremediation was occurring in the studied contaminated aquifer. Abundant residual BTEX is present in the unsaturated zone around the area of well MP2, particularly in the capillary fringe. These contaminants leached from the sediment by seepage and groundwater and are continuously transported downstream in the aquifer. The long term investigation results show that natural attenuation mechanism are occurring and causing BTEX removal from groundwater by biologically mediated degradation under anaerobic conditions. A simultaneous occurrence of nitrate, iron and sulphate reduction and methanogenese was evident at the site but no distinct redox zones were observed within the contaminant plume. At one contaminated well, a low concentration of oxygen has likewise been detected. Based on the evaluation of results, iron reduction and methanogenese are the dominant biodegradation processes for the observed part of the plume. Evidence of biodegradation include: (1) depletion of DO in the observed part of the plume; (2) production of biodegradation by-products Fe(II) and methane; and (3) production of metabolic intermediates benzoic acid and their homologs C_1-, C_2- and C_3-benzoic acid, generated by bacteria. In this study it could be shown that the measurements of TEAPs and metabolic by-products are two helpful tools to provide evidence for intrinsic biodegradation processes in groundwater at contaminated sites.

5 Long–term observations on the influence of groundwater level variation on BTEX concentrations in groundwater

Abstract

A long term study was carried out at a former gasoline filling station at the former military site Schäferhof-Süd (Niedersachsen) investigating natural attenuation and remediation in sediment and groundwater. A large residual contamination with benzene, toluene, ethylbenzene, xylene (BTEX) and petroleum hydrocarbons is present in the sediment at this locality. BTEX-concentration in the groundwater and its correlation with fluctuations of the groundwater level was monitored over a period of three years. A very dry summer (2003) was recorded during the monitoring period, resulting on site in a drop of the groundwater level to 1.7 m and a contaminant increase of BTEX concentrations from 240 µg/l to 1300 µg/l. Microbial degradation of BTEX was documented by data derived from the consumption of electron acceptors (oxygen, nitrate or sulphate) and the production of reduced products (Fe(II), methane). The detection of metabolites confirm degradation. Increasing BTEX concentrations are hence not a consequence of limited biological degradation.

5.1 Introduction

Surface spill accidents arising out of leakages involving mineral oils and derived products prove to be the greatest contaminants in groundwater these days. Especially the easily soluble aromatic compounds benzene, toluene and ethylbenzene, the three xylene isomers (BTEX) have a toxic and carcinogenic potential and endanger the quality of groundwater and consequently drinking water resources (An, 2004; Kermanshahi pour et al., 2005; Kao et al., 2006). BTEX hydrocarbons enter the groundwater by means of contamination with gasoline and jet fuel (Wiedemeier et al., 1999; Cozzarelli & Baehr, 2003; Andreoni & Gianfreda, 2007).

An active remediation cleanup involving a BTEX event proves not only to be very expensive but almost impossible when it should come to the complete removal of contaminants from the subsurface. A favoured and common practice is combining an active remediation process focussing on the source of contamination coupled together with the monitoring of the residual contamination in the subsurface (Monitored Natural Attenuation; MNA) (Wiedemeier et al., 1999; Martus, 2002; Hinspeter & Püttmann, 2003).

This is conform with regulations laid down in the Bundes-Bodenschutzgesetz (BBodSchG, 1998) i.e. achieving the natural functions of the subsurface by means of applying bioremediation processes in reducing contamination and this as sustainable restoration. Such processes have to be environmentally sound, economical reasonable and go hand in hand with technical approaches.

This particular research study with investigation area located at UST Schäferhof-Süd was carried out as part of the research project "Langzeituntersuchungen zu den Möglichkeiten und Grenzen der Nutzung natürlicher Selbstreinigungsprozesse für ausgewählte Schadstoffe am Beispiel kontaminierter militärischer Liegenschaften (FKZ 298 76 712 /02)", initiated by the Umweltbundesamt (UBA). A monitoring period of three years was forseen. The evaluation of natural attenuation and natural remediation processes in the unsaturated and saturated zones was investigated. These studies in particular included the analysis of sediment, soil air, seepage water and groundwater. A major focus of this study attention centred on the saturated zone. This zone is assumed to serve as potential discharge from the unsaturated zone for contaminants (Hettwer et al., 2006). The findings concentrate on the temporal variations of BTEX concentrations and the redox-sensitive hydrochemical

parameters in groundwater in which special emphasis was noted in seasonal groundwater level fluctuations during the observational period at the test plot. A detailed description of the investigation area can be found in chapter 2.

5.2 Characteristics of contamination at the test plot

5.2.1 Description of the test plot

The investigated test plot UST Schäferhof-Süd was in operation up until 1976 then used as a gasoline filling station. Heavily contaminated sediment was found in an area of 10 to 25 m and at a depth of 1.30 m once the subsurface filling station and the mixing station for jet fuel were demolished and removed in 1990. Concentrations of petroleum-derived hydrocarbons varied between 87 and 5450 mg/kg whereas BTEX concentrations varied between 1.5 and 109 mg/kg. After the demolition work on the buildings had concluded in 1995, the area was levelled off and covered with a water-proof plastic film. This proved necessary in order to prevent modification of subsurface contamination. The foil was removed in January 2001, so that natural sediment conditions can resume again.

Sediment samples at eight drilling areas on the test plot were taken with a total depth of 8 m below ground surface (bgs) at the beginning of the project and likewise at the end of the projects duration (Hettwer et al., 2006).

At each drilling area (diameter 1 m), the initial and final sampling can be represented as two single drillings and performed as two spatially opposite located single-drillings (liner, DN 100). The two sediment samples taken prove beneficial when comparing analytical results but also provide valuable information on local contamination inhomogenities. Figure 13 shows the detailed map of the filling station with outlines of the gasoline filling station marked as test plot along with the drilling areas.

The groundwater observation well MP2, located in the centre of the test plot, is drilled as a double level monitoring well. All four further double level monitoring wells are sunk along a transect in the direction of the groundwater flow in the upstream and downstream sections of the test plot. The data from the complete set of observation wells and the reference plot are subject of chapter 4 and referenced in Hettwer et al. (2006).

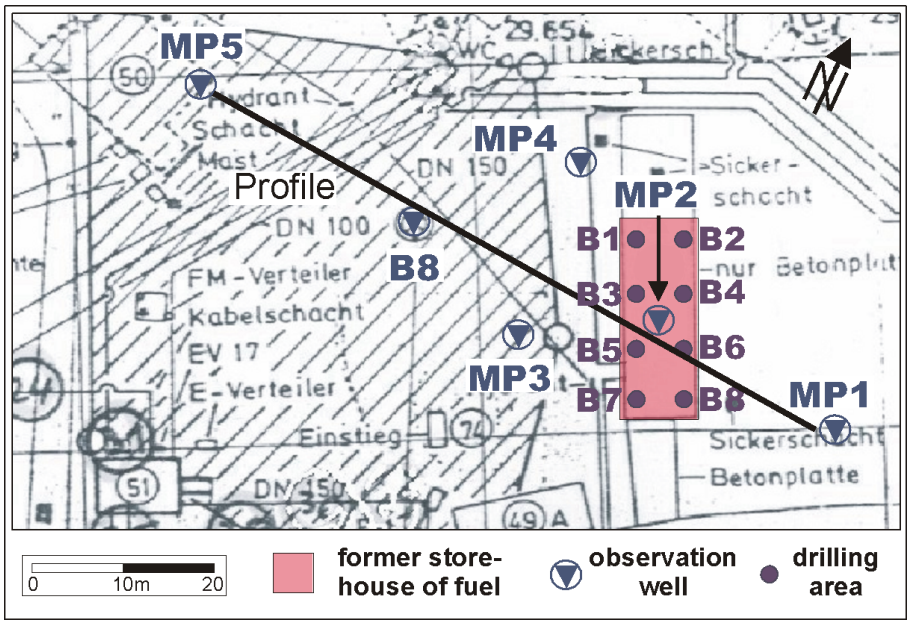

Figure 13: Map of the UST Schäferhof Süd. Shown are the test plot with the eight drilling areas and the positions of groundwater observation wells.

5.2.2 BTEX sediment contamination

Maximum BTEX concentration values of 17 mg/kg (drilling point B3/3) were recorded in analysing sediment in the unsaturated zone. In the capillary fringe, values of 450 mg/kg (drilling point B1/4) were recorded (October 2004). The saturated zone was only sampled by drillings B3 and B5 whereby maximum values of 6.7 mg/kg BTEX were detected. These findings display a vertical inhomogeneous BTEX distribution. An increase of BTEX concentrations in the capillary fringe was observed in all drillings. Deviations in BTEX concentrations were also detected in the horizontal plane with inhomogeneous contaminant distribution.

Figure 8 in chap. 4, p.48 exemplary displays the vertical distribution of contaminants in the test plot at the drilling areas B3 and B5 to a depth of 8 m. The unsaturated zone (u), the capillary fringe (c) and the saturated zone (s) are indicated. The maximum and minimum groundwater level recorded at MP2-f is likewise depicted in figure 8.

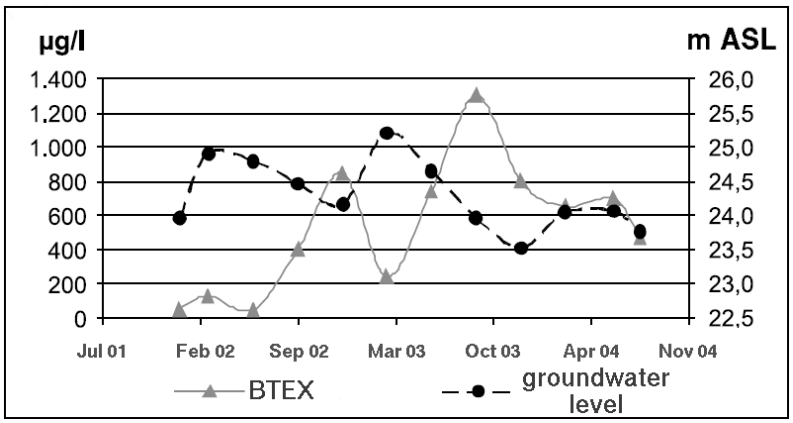

Figure 14: Chronological sequence of BTEX-concentrations and groundwater level at the observation well MP2(upper section).

5.2.3 BTEX-concentration in groundwater influenced by groundwater level fluctuations

The groundwater level was determined on a regular three month basis at the upper screened sections of the groundwater observation well MP2, located in the centre of the test plot in the investigation area. Typical seasonal fluctuations were observed within the monitoring time frame, indicated by the sinoidal hydrograph over the course of one year.

A maximum groundwater level was recorded in March 2003 with 25.5 mNN. Due to extreme low precipitation rates in the following summer, the groundwater level steadily decreased and in December 2003 a level of 23.5 mNN was noted (refer to fig. 14). An increase of the groundwater level occurred in 2004, yet that years maximum of 24.0 mNN remained below the 2002 level/max of 24.9 mNN. In conjunction with groundwater level observations, BTEX groundwater contamination observations at well MP2 (upper section) were also conducted. BTEX concentrations ranging from 48 µg/l to 1.300 µg/l were recorded. Slightly lower concentrations of 48-130 µg/l were recorded at the beginning of the first monitoring year (fig. 14). This can be explained by the fact that the natural sediment environment was largely disturbed when the test plot was covered with a water-proof plastic film and once the film was removed a natural sediment humidity could resume again. Between the years 2002 to 2004, a general increase in BTEX concentrations at the groundwater

can be observed.

It furthermore becomes apparent that a negative relationship exists between the groundwater hydrograph and contaminant concentrations in which an increase in BTEX concentrations in the groundwater and a decrease in the groundwater level has been observed. In contrast however, an increasing groundwater level is accompanied by a decrease in BTEX concentrations. This results in an detailed inverse proportional relationship between groundwater level hydrographs and BTEX concentrations at MP2 in particular over the time period between September 2002 and September 2003.

As shown in figure 8, the layer of sediment with the highest contamination concentrations is in direct contact with the groundwater flow at low groundwater levels. An increase in BTEX concentrations occurs during dry periods due to an elevated residue of contamination in the zone of groundwater fluctuations. During periods where the groundwater level rises, the surface of the groundwater penetrates to the zone of lower contaminated sediment resulting in a decrease of BTEX-concentration in the groundwater due to dilution. The zone of maximal hydrocarbon concentrations coincided with the lowest recorded position of the groundwater table at the depth of about 5-6 m. As an effect of the groundwater table fluctuations the BTEX were smeared in vertical direction. Such a strong relationship between hydrogeological settings and spatial distribution of the hydrocarbons has been also observed by Klonowski et al. (2008).

5.2.4 Hydrochemical indicators for biodegradation

Redox-sensitive parameters derived from the analysis of electron acceptors or metabolic by-products can act as indicators for biologically mediated degradation. The naturally occurring electron acceptors are dissolved oxygen, nitrate, ferric iron, sulphate and carbon dioxide and are consumed during microbial metabolism of organic contaminants such as for example BTEX (Schlegel, 1992; Wiedemeier et al., 1995, 1999). Via enzymatic catalysed redox reactions, electrons are made redundant in oxidising reactions and are resorbed as electron acceptors. During aerobe respiration oxygen (O_2) is used as an oxidising agent. In the absence of O_2 however, the following compounds can act as anaerobic electron acceptors: nitrate (NO_3^-), ferric iron (Fe^{3+}), sulphate (SO_4^{2-}) and carbon dioxide (CO_2) (Stumm & Morgan, 1970;

		MP1-f	MP2-f
BTEX µg / l (n = 12)	min	< 1	48
	med	< 1	560
	max	< 1	1300
O_2 mg / l (n = 12)	min	2.80	0.1
	med	5.25	0.2
	max	8.40	3.5
NO_3^- mg / l (n = 12)	min	24	< 1
	med	48	2
	max	62	15
Fe^{2+} mg / l (n = 12)	min	< 0,02	26
	med	0,07	32
	max	1,08	79
SO_4^{2-} mg / l (n = 12)	min	22	10
	med	27	21
	max	34	58
CH_4 µg / l (n = 7)	min	< 10	< 10
	med	< 10	1300
	max	< 10	1500
carboxylic acids µg / l (n=5)	min	< 1	52
	med	< 1	161
	max	2.2	700

Table 6: Concentrations of redox-sensitive parameters and metabolites (sum of benzoic acids)

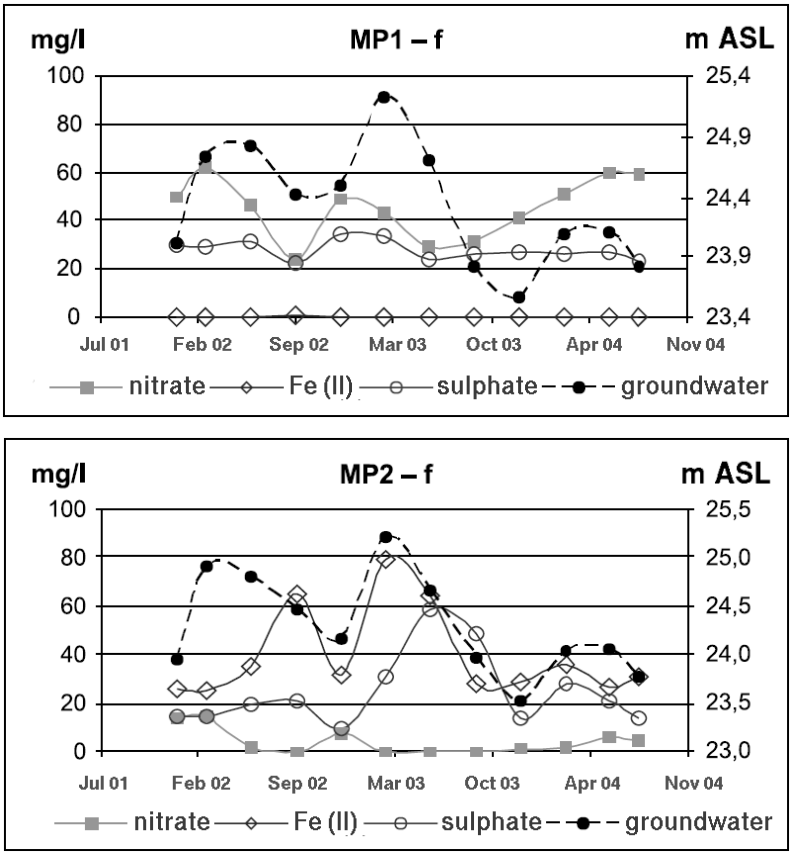

Figure 15: Hydrochemical parameters measured at the observation wells located in the upstream and in the test plot

Wisotzky & Eckert, 1997; Heider et al., 1999; Spormann & Widdel, 2000; Beller, 2000; Phelps & Young, 2001). The redox potential and energy efficiency is reduced from NO_3^- to CO_2.

At the UST Schäferhof-Süd, characteristical variations of the aforementioned parameters can be observed when comparing results obtained from the contaminated observation well MP2 and the uncontaminated observation well MP1 (see table 6). In figure 15 the hydrochemical parameters nitrate, ferrous iron and sulphate, as well as the groundwater level for the groundwater observation well MP1-f and MP2-f are displayed.

In the upstream sector, the mean nitrate concentrations are 48 mg/l, 27 mg/l for sulphate and less than 0.1 mg/l for ferrous iron. In the test plot of the investigation

area, nitrate has almost been completely removed. Likewise, ferrous iron mean concentrations of 32 mg/l show a significant increase at the well MP2. These results correlate with the BTEX concentrations in groundwater at the observation well MP2-f, due to the fact that biological degradation is enhanced by an increase in organic material. Also the reduction of sulphate is observed, even if not completely.

Figure 15 also shows that only small variations of sulphate and ferrous iron is evident at groundwater observation well MP1 due to the dynamics of the groundwater level. In the upstream sector, the nitrate concentrations correlate with the groundwater level. This can be explained by a high NO_3^- contribution in particular during winter and spring seasons arising from agricultural seepage water. This situation involving nitrate concentration changes course in the test plot. As of September 2002, sulphate and ferrous iron concentrations display a clear correlation with the groundwater level. The NO_3^- concentration decreases significantly due to the consumption of nitrate. In comparison to the observation well located upstream, only a small increase in December 2002 and June 2004 can be noted. The increase in the sulphate concentration can be explained by variations of the source material.

The first two meters of the subsurface was filled using medium grained sand containing building rubble of varying proportions, well known for its sulphate emissions in seepage water. An increase in precipitation leads to the elution of SO_4^{2-} in seepage water coming from this filling material (Hornbruch et al., 2007). The increase of Fe(II) on the other hand can be traced solely back to the biological decomposition of hydrocarbons. An increase in the groundwater level ultimately leads to the ground water flowing through the sediment body thereby leading to the enlargement of the streamed sediment body. The volume of sediment available for ferrous iron reduction is hence enlarged. Biological decomposition caused by iron reduction is thus largely enhanced by an increase in the groundwater level. Methane concentrations of 1.300 µg/l (median) argue for microbial degradation of hydrocarbons under primary anaerobic settings.

An additional parameter used in recent years as an indicator for NA in the scope of MNA-studies, is the detection of aromatic carboxylic acids (Beller, 2002). The mineralisation of the contaminants by microorganisms initially starts under the consumption of oxygen. But due to oxygen´s low solubility in groundwater, oxygen is only available to a limited extent. Therefore, contaminant degradation occurs

mainly under anaerobic conditions. In the detection of specific metabolic single compounds, conclusions can be drawn as to initial compounds found in contaminated groundwater. Benzoic acid is for example an intermediate product of toluene in the biological degradation process (Biegert et al., 1996).

Benzoic acid can also be produced in sediment by several other biological processes and is not chemically stable. Hence, the use of benzoic acid on its own as a monitoring detector is not the best parameter to use in the monitoring of degradation processes of aromatic hydrocarbons. Alkylated benzoic acids on the other hand give a good insight on the degradation of anthropogenic introduced contaminants (motor gasoline, jet fuel). Aromatic acids are formed by the anaerobic hydroxylation of the methyl group. Derivatives of benzylsuccinate are generated as intermediate products, as evident in anaerobic toluene degradation (Biegert et al., 1996). Methylbenzoic acid is an intermediate product in C_2-benzene degradation. Analogue are C_3- and C_4-benzene the parent hydrocarbons for the C_2- and C_3-benzoic acids (Beller, 2000; Namocatcat et al., 2003).

Furthermore, benzylsuccinic acid and methylbenzylsuccinic acid isomers are proposed as distinct indicators of anaerobic toluene and xylene metabolism. These succinic acids have no commercial or industrial use and having an unequivocal relationship to parent hydrocarbons (Beller, 2002; Reusser et al., 2002; Namocatcat et al., 2003). The isomers are referred to "sum of isomers" in table 5. The detection of organic acids therefore provides direct proof for active biological degradation of gasoline and jet fuel compounds at the time of sampling.

The groundwater was sampled twice a year in order to analyse metabolites using mass spectrometry. Details on the sampling procedure and the analysis technique are referred to in chapter 3. During the complete monitoring period, single compounds benzoic acid, isomers of the methylbenzoic acid and isomers of the C_2-and C_3-benzoic acid were detected. These isomers are referred to later and in table 6 in their summed parameter as sum of benzoic acids. Levels of a maximum of 2.2 µg/l benzoic acid have been recorded at the groundwater observation well MP1-f representing a natural background concentration. The carboxylic acid concentrations of up to 700 µg/l (June 2003) at the test plot are significantly increased. The sum of benzoic acids comprises mainly of isomers of the dimethylbenzoic acid and the isomers of trimethylbenzoic acids whereby the benzoic acid only represents approx.

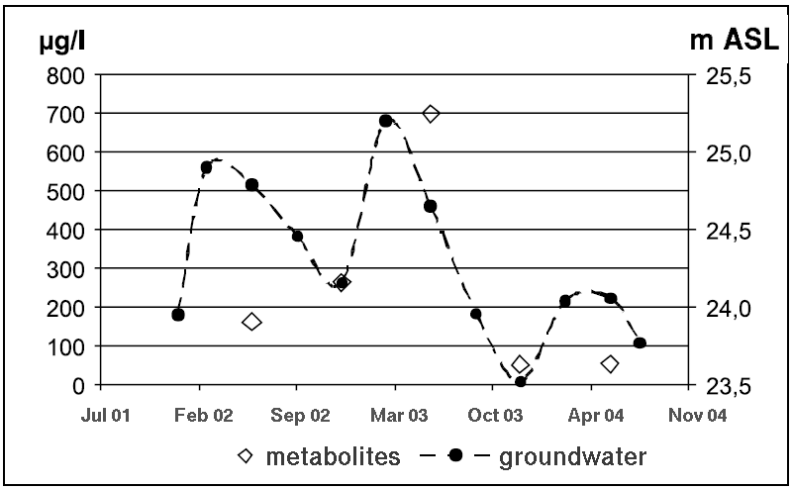

Figure 16: Correlation of groundwater level and detected metabolites (sum of the compounds benzoic acid, isomers of methylbenzoic acid, dimethylbenzoic acid and trimethylbenzoic acid) in groundwater at MP2-f.

1% of the total concentration. Detailed information on these relationships can be found in chapter 4.

In table 6 are given the concentrations of carboxylic acid at groundwater observation well MP2-f showing that great deviations exist. A correlation between the groundwater level and the concentration of carboxylic acids however evolves towards the end of 2002 (compare with fig. 16). An increasing groundwater level leads to a significant increase in the metabolites concentration as was detected in June 2003. The retreat of the groundwater level in the following winter at groundwater observation well MP2-f resulted in a decrease in the carboxylic acids concentration up to the minimum concentration of 52.4 µg/l.

5.3 Conclusions

The findings show that increased BTEX concentrations in groundwater are not a consequence of limited biological degradation at the location UST Schäferhof-Süd. Parallel to an increase in contaminant concentrations, changes in redox sensitive hydrochemical parameters were likewise detected in the groundwater, thereby clearly indicating active biological metabolism of organic material by microorganisms. This

was confirmed by the detection of metabolites.

Due to the residual contamination in the unsaturated zone can be observed an increased solution of BTEX from the unsaturated zone to the groundwater during periods of enlarged precipitation, which causes an increase of the groundwater level. The correlation between the metabolite concentration and the groundwater level is hereby confirmed as depicted in figure 16.

For the monitoring of natural degradation and retention processes at localities with residual contaminations in the unsaturated zone two major issues become apparent. These being the focus on the lengthy duration of monitoring period and the influence of groundwater level fluctuations. The following conclusions can be drawn from the findings of this study and their applicability in association with natural attenuation:

1. Misleading conclusions can be made in respect to NA on the evaluation of the biological degradation of organic contaminants in groundwater particularly during the course of short term observations and monitoring periods e.g. periods less than one year. As observed at the beginning of monitoring at the investigation area Schäferhof-Süd, a decrease in the BTEX concentration in groundwater can suggest a continuous approximation towards successful remediation. An increase however in the contaminant concentration in groundwater cannot be interpreted as a failure of the effectiveness of NA. In that case, the application of useful parameters, such as the detection of electron acceptors and metabolites must prove biological degradation. It has become evident in this study, that long term monitoring is essential for the clarification of expected contaminant concentrations and variations in groundwater.

2. The most important factor in this study is the influence of groundwater level fluctuations. The groundwater level fluctuations, natural degradation and retention processes essentially influence BTEX concentrations in the groundwater. Groundwater level fluctuations are by far a stronger influence than the influence of biological degradation.

The localisation of the contamination in the unsaturated zone is essential in order to be able to explain the variations of BTEX concentration in the groundwater.

The findings and results of this study indicate that the influence of groundwater level fluctuations must be included as a parameter in modelling calculations in order to determine degradation rates. Currently available models (e.g. Bioscreen-Natural-Attenuation-Decison-Support-System) do not incorporate groundwater level variations as a relevant parameter.

6 Biodegradation of BTEX in a contaminated aquifer under iron reducing conditions associated with fractionation of iron isotopes

6.1 Abstract

Biodegradation of monoaromatic hydrocarbons under anaerobic conditions in the subsurface of contaminated sites is accompanied by reduction of electron acceptors. In this study, first field data are presented on isotopic fractionation of the electron acceptor Fe(III), due to biologically mediated reduction of Fe(III) to the water-soluble Fe(II) at a BTEX contaminated site. Both groundwater and sediment samples were analysed with respect to their Fe isotopic compositions using high mass resolution MC-ICP-MS (Multi Collector-Inductively Coupled Plasma-Mass Spectrometry).

Observation wells were installed upstream, downstream and within the source area of the BTEX contaminated site. The δ^{56}Fe-values of groundwater samples taken from observation wells located downstream of the source area displayed delta values of around -0.20‰ and were isotopically lighter than δ^{56}Fe-values obtained from groundwater in the uncontaminated well with δ^{56}Fe-values of 0.01‰. Additionally, two sediment profiles of 8 m depth were drilled in the source area of the contamination. The Fe isotopic composition of most parts of the sediment profile was similar to the Fe isotopic composition of uncontaminated groundwater. But some sediment samples in the profile were found to be significant isotopically heavier, especially in the depth range of the unsaturated zone and the capillary fringe. The δ^{56}Fe-value for sediment samples ranged therefore between 0.02‰ and 0.25‰. Thus, a significant iron isotope fractionation can be observed between sediment and groundwater downstream of the BTEX contamination. The maximum observed difference for δ^{56}Fe was up to 0.46‰ between sediment and groundwater.

6.2 Introduction

Leakages of gasoline and jet fuel from underground storage tanks or surface spill accidents belong to the prevalent challenges of sediment and groundwater cleanup operations today. Due to their water solubility BTEX were the dominating groundwater contaminants of these spills before the introduction of oxygenates such as methyl-*tert.*-butylether (MTBE). Since oxygenates were not added to gasoline in Europe until 1995, the problem with gasoline and jet fuel related groundwater contaminations that occurred before 1995 is preferentially related to BTEX aromatics.

NA is increasingly accepted as an option for the handling of BTEX contaminated aquifers, provided that the effectiveness of natural degradation processes of the hydrocarbons have been proven at the contaminated site. Therefore are BTEX commonly included in groundwater monitoring programs at such sites (e.g. Wiedemeier et al., 1999). The steps and processes during biodegradation are still under investigation and new methods to document and monitor these processes are desired (e.g. Beller, 1995; Wiedemeier et al., 1999; Spormann & Widdel, 2000; Chakraborty & Coates, 2004).

Measurable changes in chemical parameters of groundwater in a contaminated area can indicate intrinsic processes of biodegradation. But no single parameter is considered sufficient to demonstrate the occurrence of intrinsic bioremediation in the field (NRC, 1993). Instead, multiple strategies are required to prove the effectiveness of microbial attenuation of hydrocarbons in the subsurface.

Methods like the measurement of electron acceptors (e.g. Schreiber et al., 2004), the carbon isotopic composition of individual hydrocarbons (e.g. Meckenstock et al., 1999; McKelvie et al., 2005), the analysis of metabolic by-products in the contaminant plume (e.g. Beller, 1995, 2000; Martus & Püttmann, 2003; Gödeke et al., 2006) can act as indicators for this biologically mediated degradation. Naturally occurring electron acceptors are dissolved oxygen, nitrate, ferric iron, sulphate and carbon dioxide and are used in microbial metabolism of organic contaminants. The effectiveness of the reduction of oxygen, nitrate and sulphate and production of Fe(II) and carbon dioxide as degradation processes for hydrocarbons can be monitored by measuring the depletion of the electron acceptors in the contaminated area, relative to non-contaminated upstream groundwater (Baedecker et al., 1993; Heider et al., 1999; Beller, 2000; Spormann & Widdel, 2000; Phelps & Young, 2001;

Farhadian et al., 2007).

Fe(II) is one of the metabolic by-products of anaerobic respiration of ferric iron and increasing concentrations in groundwater can confirm the occurrence of Fe(III) reduction (Wiedemeier et al., 1999). However, the reaction product Fe(II) is higly reactive and can be removed from the groundwater for example by formation of iron monosulfides. When sulphate reduction and iron reduction take place simultaneously, Fe(II) and hydrogen sulphide are at least partly removed from the solution by rapid precipitation of immobile iron sulphide minerals (Ulrich et al., 2003). This precipitation therefore makes the measurement of Fe(II) concentrations in the groundwater to an inappropriate tool for the assessment of the extent of iron reduction as a degradation process for hydrocarbons. Moreover, the reaction educt Fe(III) is only water soluble after complexation by organic ligands (e.g. Brantley et al., 2004; Wiederhold et al., 2006). For both reasons the assessment of iron reduction in biodegradation processes only by the measurement of Fe concentration in groundwater is critical.

Several studies about the geochemical behaviour of the light stable isotopes of S, H, N, O and C have contributed substantially to the unterstanding of the inorganic and biological processes in the subsurface (Schidlowski et al., 1983; Meckenstock et al., 1999; Hayes, 2001; Meckenstock et al., 2004a; Bugna et al., 2005). In recent years, proceedings of methods became available, which readily permit the accurate and precise determination of Fe isotope fractionation. This provides geochemists with a useful tool to investigate the biogeochemistry of Fe in low temperature environments (e.g. Bullen & McMahon, 1998; Beard et al., 1999; Brantley et al., 2001; Bullen et al., 2001; Johnson et al., 2002; Beard et al., 2003; Anbar, 2004; Brantley et al., 2004; Beard & Johnson, 2004; Johnson et al., 2004; Weyer & Schwieters, 2003; Arnold et al., 2004; Teutsch et al., 2005; Weyer et al., 2005; Wiederhold et al., 2006).

The metabolic processing of iron reduction involves a number of steps, such as transport across membranes and uptake by enzymes that may produce a measurable Fe isotopic fractionation in sediment and groundwater (Beard et al., 1999). Experiments have proved biological fractionation of Fe isotopic composition through Fe reducing bacteria which are able to use the abundant Fe(III) in minerals as an energy source (Beard et al., 1999; Brantley et al., 2001; Beard et al., 2003). Bullen

& McMahon (1998) describe an example of using this iron isotope system for a mass balance calculation to measure the extent of Fe(III) reduction in sediment. An isotopic fractionation by dissimilatory Fe reducing bacteria can cause isotopic variations of 1.3‰ to 1.4‰ when Fe(II) is released to solution during the reduction of Fe(III) from different minerals (e.g. ferrihydrite, hornblende and goethite). Isotope fractionation during processes like precipitation (Bullen et al., 2001), adsorption (Teutsch et al., 2005) and by kinetic or equilibrium fractionation effects (Johnson et al., 2002; Anbar, 2004; Johnson et al., 2004; Beard & Johnson, 2004; Butler et al., 2005) have also been observed.

Insoluble Fe(III) oxides, which are abundantly present in shallow aquifers, can be mobilized via complexation by organic ligands (Lovley et al., 1994b). The formation and subsequent detachment of Fe(III) ligand complexes represent the rate limiting step for ligand controlled dissolution (Zinder et al., 1986). Thereby the bioavailability of Fe(III) is increased drastically and biodegradation of aromatic hydrocarbons such as toluene and even benzene is advantaged (Lovley et al., 1994b). Ligand-controlled dissolution experiments of Brantley et al. (2004) and Wiederhold et al. (2006) resulted in significant fractionation of iron isotopes. A significant abiotic fractionation of iron isotopes was observed during dissolution of hornblende (Brantley et al., 2001, 2004) in the presence of different organic ligands such as oxalic acid, acetic acid, citric acid and the siderophore desferrioxamine mesylate. This is also the case in the reductive dissolution of goethite (Wiederhold et al., 2006).

In this chapter, field data are presented which demonstrate fractionation of the iron isotopes due to reduction of Fe(III) in a BTEX contaminated aquifer. Samples of sediment and groundwater were analysed in order to identify different iron isotopic signatures within these reservoirs.

6.3 Experimental Section

6.3.1 Sampling for characterisation of contamination by classical geochemical analyses

Over a period of three years (2002-2004), groundwater samples for hydrochemical analyses were taken at regular intervals, four times a year, from the double level monitoring wells MP1–MP5 and one full screened well B8 and analysed. The

sampling of the full screened well was finished and substituted by sampling of the double level wells in June 2003. For localisation of the observation wells at the UST Schäferhof Süd see figure 1 in chapter 2 on page 24. Sediment samples were taken from the drilling wells B3 and B5 in the area of the former fuel storehouse with various depth profiles (see fig. 13 in chap. 5 on p. 67).

The sampling of groundwater and sediment is detailed described in chapter 4.2.1.

6.3.2 Sampling for Fe isotope analysis

During the groundwater sampling in June 2003, the samples for Fe isotope measurements were taken from the wells MP1, MP2, MP4, MP5 (upper section) and from the full screened well B8. The sampling procedure was analogous to that previously described for geochemical analysis in chapter 4.2.1. 1l of groundwater was taken, cooled and stored in dark glass bottles during transportation to the laboratory.

Sediment samples were taken from drilling B3 and B5 (both 8 m) as aforementioned and placed in headspace glasses. Samples were taken from one of the double face drillings (B3/3 and B5/3). The sampling for the Fe isotope study was performed in the final two years of the project, enabling the presentation of a complete data set.

6.4 Analytical methods

Groundwater samples were shipped for BTEX, NO_3^-, SO_4^{2-} and Fe^{2+}, testing to a commercial laboratory. BTEX analyses of groundwater and sediment samples were carried out following DIN 38407-F9 (equivalent with EPA Method 8020). Details according to this method are described in chapter 4.2.2.

Nitrate and sulphate concentrations of groundwater were determined by use of photometric methods and the MDL for both was 1 mg/l. Analyses of Fe^{2+} was performed by atomic absorption spectroscopy (AAS) (MDL 0.02 mg/l). The analysis of total iron content in sediment samples was undertaken using the method DIN EN ISO 11885 (equivalent with EPA Method 3051).

Fe isotope measurements were performed using the Thermo Finnigan Neptune MC-ICP-MS at the University Frankfurt a.M., Germany. The Neptune is a double-focusing multiple collector ICP-MS, which has the capability of high mass resolution

measurements in multi collector mode (Weyer & Schwieters, 2003). Sample preparation was performed in a clean-room laboratory.

50 ml of each groundwater sample was dried in Savillex vials on a hot plate. Due to the low ferrous iron content of uncontaminated groundwater, it was necessary to use a larger volume of the uncontaminated sample obtained from MP1 (500 ml).

About 50 mg of each sediment sample were used for Fe isotopic analysis. The sediment samples and residual groundwater samples were treated as follows: Firstly, the samples were treated with concentrated HNO_3 and left overnight on a hot plate in a closed vial, enabling most organic matter to oxidize. Once dried, the sediment samples were completely digested using a mixture of concentrated HF/HNO_3 with a ratio of 3:1 and again dried out.

During the next step the samples were redissolved in 7M HCl with a small amount of H_2O_2. This step is required to ensure that the iron remained as Fe(III). The samples were dried again and further redissolved in 2 ml of 7M HCl with H_2O_2. This 2 ml solution was loaded onto an anion exchange column (BioRad 2 ml Columns, BioRad AG 1x8 resin). The prepared resin had been washed three times with 0.5M HCl and cleaned and conditioned once with 7M HCl. Matrix elements were separated from Fe in the sample by rinsing the loaded columns with 7M HCl (30 ml). Fe was then eluted in 0.5M HCl (10 ml).

The entire sample preparation was performed according to standard procedure of the lab, which was established by experiments and thorough reapplication by Arnold et al. (2004) and Weyer et al. (2005). The accuracy and precision of the method was further checked by continuous measuring it against several international and inhouse standards, such as BIR-1, AKA-4 and FeOOH-7. Our results correlate with those of previous studies (Weyer & Schwieters, 2003).

Sample and control solutions were diluted to 5 ppm total Fe concentration for measurement. A 3 ppm Cu-standard (NIST 976) was added to each sample and control prior to analysis. The $^{65}Cu/^{63}Cu$ ratio of the control, which was measured together with the Fe isotope composition of the sample in a dynamic mode, combined with sample-standard bracketing, was used to ensure against any instrumental mass bias. All measurements were operated in high resolution mode to eliminate isobaric interference of polyatomic ions $^{40}Ar^{14}N^+$, $^{40}Ar^{16}O$ and $^{40}ArOH^+$ on ^{54}Fe, ^{56}Fe and ^{57}Fe, respectively (Weyer & Schwieters, 2003). Details about the mea-

suring protocol are described by Arnold et al. (2004) and Weyer et al. (2005). All samples were measured at least twice. Fe isotope values reported here, are the means of these replicate measurements and reported error bars are based on replicate measurements of samples. Iron isotope compositions are reported relative to the international Fe standard IRMM-014 using the delta notation:

$$\delta^{56}\mathrm{Fe}_{\mathrm{sample}} = \left(\frac{(^{56}\mathrm{Fe}/^{54}\mathrm{Fe})_{\mathrm{sample}}}{(^{56}\mathrm{Fe}/^{54}\mathrm{Fe})_{\mathrm{IRMM-014}}} - 1 \right) \cdot 1000$$

6.5 Results

6.5.1 BTEX in groundwater

The groundwater sample collected at the well MP1, located upstream from the source of the contamination, was free from any contaminants and was thus used as reference for the other localities. In groundwater flow direction a increase of BTEX concentrations in the upper screened wells was observed (fig. 17). In the lower screened wells, with the exception of MP5-t, no BTEX could be detected. In well MP5 very high concentrations of BTEX were consistently measured. The median value (n=12) of BTEX concentrations in the upper well of MP5 were 2850 µg/l and in the lower well 3100 µg/l. The highest concentration was measured in groundwater samples taken from the upper well MP5 with up to 6.900 µg/l of BTEX. In contrast, in MP2 only 560 µg/l in median were detected. At the observation well B8, which is a full-screened well, the groundwater provided a composite sample from an 8 m screen range. This explains the lower concentration of BTEX in the groundwater at B8 (715 µg/l) compared with the upper sections of the neighbouring wells MP4 and MP5.

The observed distribution of BTEX concentrations indicates that the available wells cover only a part of the BTEX plume originating from the former fuel storehouse. A further source of BTEX must be present downstream particularly in the area of MP5. This is supported by the observed increase of BTEX concentrations in flow direction (fig. 17).

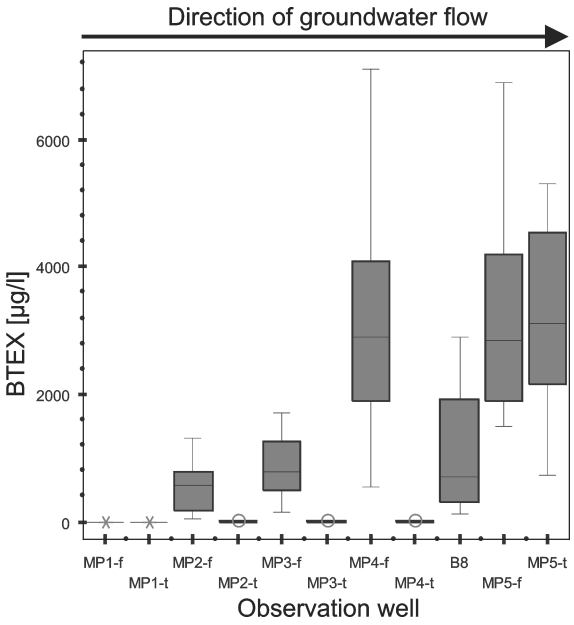

Figure 17: Boxplots of BTEX-concentrations in groundwater (measured during 12 sampling campaigns in the time from Jan. 2002 to Sep. 2004). Boxplots show clearly the variation limits of concentrations at individual points and variations of the chemical parameters in groundwater flow direction are identifiable. There is no declaration on a time-based variation. In groundwater flow direction an increase of the contaminants BTEX is observed in the upper section. Contamination of the lower groundwater section is only observed at MP5. (o = outlier; * = extreme value)

6.5.2 BTEX in sediment

The results of the investigation of BTEX contamination of sediment samples taken from profiles at the drilling points B3 and B5 are shown in figure 8. In the unsaturated zone a maximum value of 17 mg/kg (drilling point B3/3) was measured. The BTEX concentration increased to 120 mg/kg (drilling point B3/3) in the capillary fringe (5-6 m depth) while in the saturated zone below a maximum value of 6.7 mg/kg was detected (drilling point B3/4). These data indicate a very heterogeneous distribution of BTEX in the vertical sediment profiles. The significant increase of BTEX contamination in the capillary fringe is clearly shown in figure 8. This is due to the fair solubility of BTEX in water.

In figure 8 the maximum and minimum value of groundwater levels for the period

of monitoring is marked. The highest contamination is detected at a depth range between 5 and 6 meters. The maximum coincides with the lower level of the groundwater surface during the monitored period. Due to seepage water, the contaminants migrate to the capillary fringe and to the saturated zone and become partly dissolved in groundwater. This is consistent with the description of Dror (2002), that the amount and quality of the seepage water is one of the crucial factors for the fate and behaviour of volatile petroleum hydrocarbons in the sedimentary environment.

6.5.3 Fe(III) reduction due to biodegradation of BTEX

Results from the investigation of electron acceptors in groundwater taken from observation wells are shown in table 5on page 53. The data indicate the occurrence of anaerobic conditions within the source area and downstream area. Thus, anaerobic biodegradation can be expected to be the dominant biodegradation process within the source and downstream area (compare chap. 4).

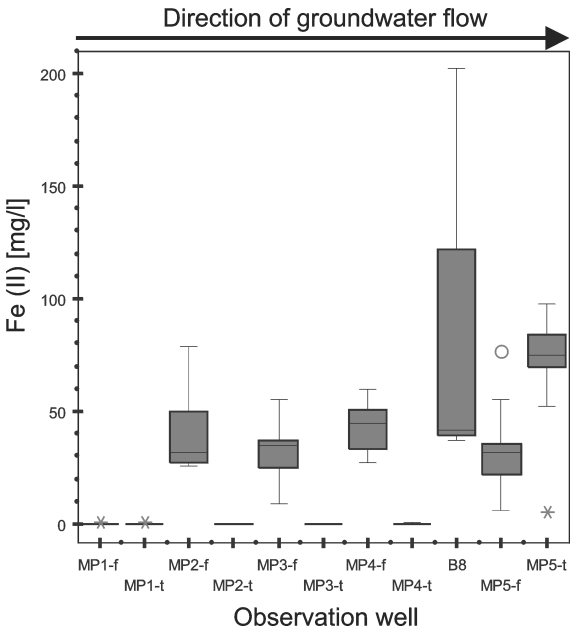

Figure 18: Boxplots of Fe(II)-concentration in groundwater (n=12). At BTEX contaminated wells elevated concentrations of Fe(II) are obtained (compare fig. 17).

The Fe(II) concentrations in groundwater from the upper screened observation

wells increase in flow direction (fig. 18). This figure shows an aprupt rise of Fe(II) concentration in groundwater from the source of contamination at well MP2. At well MP1 the mean value of ferrous iron is very low (0.15 mg/l). In contrast, at well MP4 a mean value of 43 mg/l is detected. In the lower well of MP5, elevated concentrations of ferrous iron were recorded (74 mg/l), whereas the other lower screened wells showed very low concentrations of Fe(II) in groundwater, which are comparable with concentrations measured in groundwater from MP1. At the full screened well B8 large variations of concentrations were detected with a minimum value of 20 mg/l and a maximum value of 202 mg/l. But from this well only four samples were taken, compared to the double level monitoring wells, where twelve samples within the monitoring time span were taken. Therefore, the uncertainty of the data obtained at the well B8 may be higher compared to the other wells. In figure 19 the results of analyses of BTEX and Fe(II) in groundwater samples collected within one monitoring year (Sep.2003–Aug.2004) at the observation wells MP1, MP2, MP3, MP4 and MP5 (upper and lower section) are included. The diagram shows that the concentration of Fe(II) correlates largely with the concentration of contaminants. Exeptionally high concentrations of BTEX (5300-6900 µg/l)were observed at the observation wells MP4 and MP5 (upper section) due to seasonally low levels of the groundwater (Gaab et al., 2007). These data points are marked by open dots and are excluded from the regression line shown in figure 19.

6.5.4 Fe-Isotope composition

Initially the Fe isotope measurements were focussed on the isotope composition of the individual groundwater observation wells. As shown in table 7 and figure 20 the iron isotope composition of groundwater ranges between 0.01‰ and -0.21‰ (mean values) at the study site. This variation is not very distinctive, nonetheless the variations are larger than the long-term reproducibility for water samples, which is greater than 0.10‰ for δ^{56}Fe, 2SD.

The downstream evolution from a positive to a negative value is also evident (fig. 20). At the observation well MP2 a value of 0.01‰ and at MP4 a value of -0.21‰ is measured. The groundwater samples which were taken from well MP1 and MP2 have a similar value with δ^{56}Fe close to zero. All other groundwater samples in the contaminated area of the studied site have negative δ^{56}Fe-values.

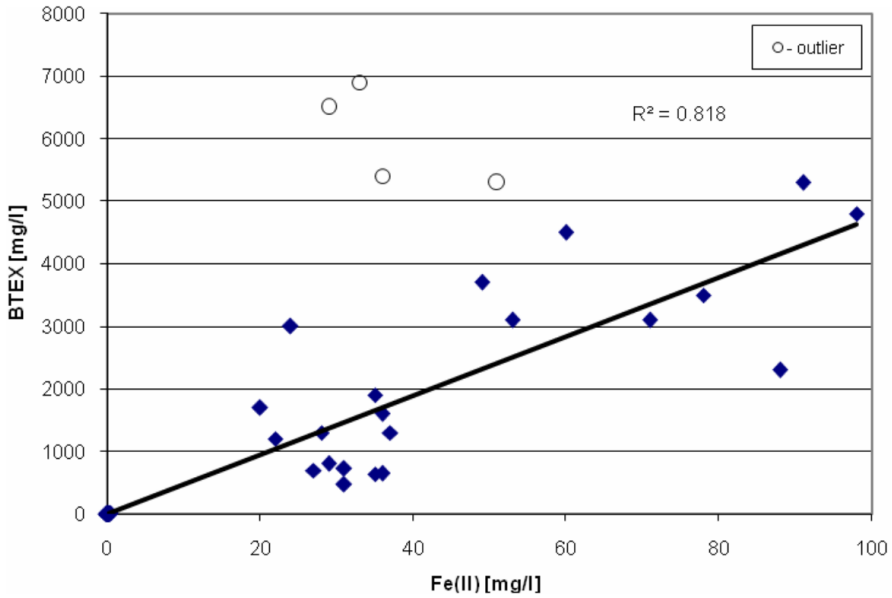

Figure 19: Correlation of BTEX concentrations and Fe(II) in groundwater, sampled in the time span from Sep. 2003 to Aug. 2004 at the observation wells MP1, MP2, MP3, MP4 and MP5 (n=50). Four data points (open dots) are excluded from the regression since the very high BTEX concentration in these samples resulting from the low water table in wintertime 2003/04. The regression line indicates increasing Fe(II) concentrations together with increasing BTEX concentrations due to biologically mediated reduction of Fe(III).

To explore the variation of the isotope composition of sediment in contrast to groundwater additionally sediment samples from the drilling profiles B3 and B5 have been analysed. The total iron concentration of sediment samples varies between 1800 mg/kg and 3500 mg/kg (tab. 27). The range of δ^{56}Fe within the profiles is small and both show similar values at comparable layers of the subsurface (fig. 21). The maximum δ^{56}Fe value in B3 is 0.25‰ and the minimum value 0.06‰ (tab. 7). At B5 a maximum of 0.19‰ and a minimum value of 0.02‰ is measured. The δ^{56}Fe data obtained from sediment samples have all positive values - with the exception of one outlier in B3 - and thus are significantly distinct from results of the groundwater testing. Both profiles are shown in figure 21 and display sligtly different variations of the δ^{56}Fe values with depth. However, beetwen 2-3 m to the depth in the unsaturated zone both profiles show their maximum positive value.

Table 7: Data of Fe content and isotopic value of sediment and groundwater samples.

sample		**Fe total** [mg/kg]* [mg/l]	δ56 **Fe**(‰) mean values
drilling	B 3/3 - 1m		0.06
	B3/3 - 2m		0.08
	B3/3 - 3m		0.25
	B3/3 - 4m		(*-0.05*) - outlier
	B3/3 - 5m		0.08
	B3/3 - 6m		0.09
	B3/3 - 7m		0.17
	B3/3 - 8m		0.16
	B5/3 - 1m	3500	0.12
	B5/3 - 2m	2300	0.09
	B5/3 - 3m	2800	0.19
	B5/3 - 4m	2000	0.12
	B5/3 - 5m	1500	0.09
	B5/3 - 6m	1800	0.04
	B5/3 - 7m		0.06
	B5/3 - 8m		0.02
groundwater	MP1-f	0.04	0.01
	MP2-f	64	0.01
	MP4-f	31	-0.21
	B8	41	-0.16
	MP5-f	58	-0.21

* Fe measurements in sediment were done on samples from drilling point B7, see figure 13 for location

The outlier in B3 could be explained by small-scale heterogeneities in the capillary fringe.

6.6 Discussion

A rapid onset of biological activity in the subsurface of a site contaminated with fuel hydrocarbons is well-known. The metabolic pathways of degradation of fuel constituents at contaminated sites are in the focus of research since about 15 years (e.g. Beller, 1995; Wiedemeier et al., 1999; Cozzarelli et al., 2000; Meckenstock

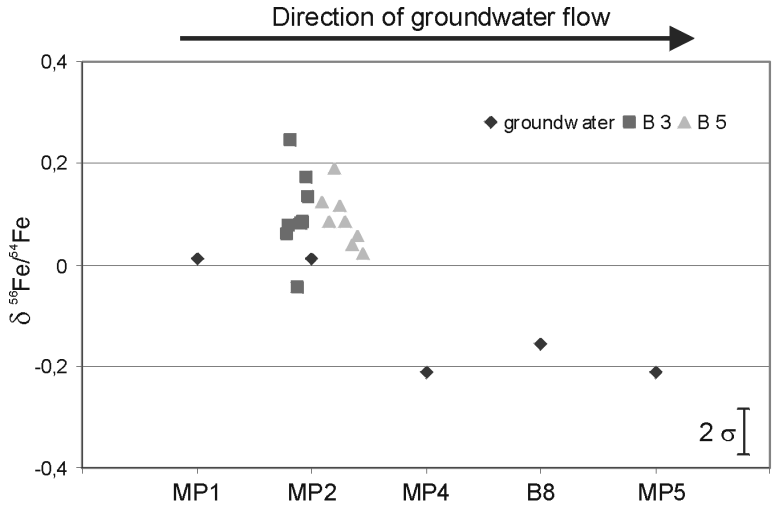

Figure 20: Fe isotope composition of groundwater and sediment samples (mean values). Sediment samples and the groundwater taken from observation well MP1-f (uncontaminated) and MP2-f display predominantly positive isotope values, whereas the contaminated groundwater taken from wells MP4-f, B8 and MP5-f in flow direction show negative values.

et al., 2000; Beller, 2002; Gieg & Suflita, 2002; Martus & Püttmann, 2003; Jahn et al., 2005). Wherever biologically mediated degradation of BTEX is observed, a depletion of the terminal electron acceptors dissolved oxygen, nitrate and sulphate and furthermore increased concentrations of Fe(II) in groundwater are reported (Wiedemeier et al., 1999; Tuccillo et al., 1999; Schreiber et al., 2004, e.g.).

Oxygen has a low aqueous solubility of approximately 8-10 mg/l in groundwater. It is rapidly consumed by aerobic bacteria when groundwater is contaminated with petroleum hydrocarbons. As a consequence, anaerobic conditions evolve quickly in the groundwater system. As shown by Wiedemeier et al. (1999), Heider et al. (1999) and Beller (2000) anaerobic biodegradation is the most significant process for the removal of BTEX from groundwater. The contribution of nitrate and sulphate reduction to the biodegradation processes can be easily assessed by measuring the depletion of these electron acceptors in the groundwater relative to the uncontaminated groundwater upstream from the contamination source. When Fe(III) is used as an electron acceptor during anaerobic biodegradation of organic carbon, it is reduced to Fe(II), which is soluble in water. Due to increasing percentages of BTEX

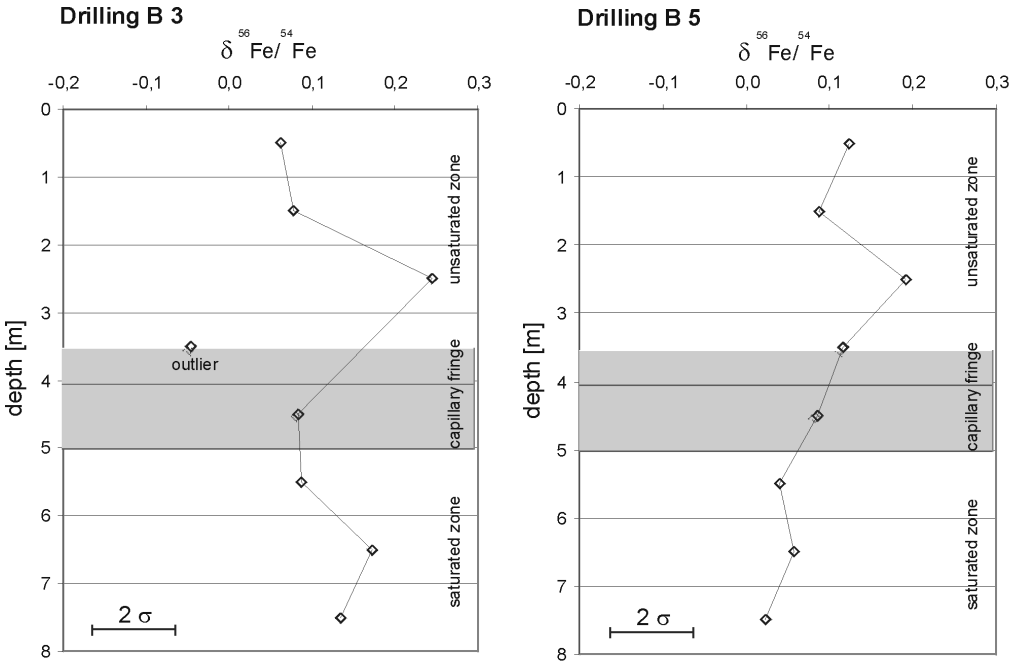

Figure 21: Fe isotope composition of sediment samples obtained from drilling profiles B3 and B5. Each value represents one meter of mixed sediment. All values are positive, except for one outlier in B3.

in groundwater flow direction, the reduction of ferric iron is stimulated. In Heider et al. (1999) the chemical reaction for the anaerobic bacterial toluene oxidation coupled to the reduction of the electron receptor Fe(III) is shown exemplary:

$$C_7H_8 + 94\ Fe(OH)_3 \longrightarrow 7\ FeCO_3 + 29\ Fe_3O_4 + 145\ H_2O$$

$$\Delta G^\circ = -3398 kJ\ (mol\ toluene)^{-1}$$

For bacteria, the energy efficiency of Fe(III) reduction is only slightly lower compared to nitrate reduction.

Analyses of sediment samples taken from the hydrocarbon-contaminated site near Bemidji (Minnesota, USA) have shown a distinctive depletion of Fe(III) in the sediment at the area of the anoxic plume. At this site the link between microbial reduction of Fe with the degradation of the hydrocarbon contaminants has been proven (Tuccillo et al., 1999). At the study site Schäferhof-Süd a positive correlation

between the contaminant concentration (BTEX) and the concentration of Fe(II) in groundwater is also recognised (see fig. 19).

Additionally the iron isotopic composition of groundwater shows a fractionation in flow direction (fig. 20). The groundwater obtained from MP1 and MP2 provided δ^{56}Fe values around zero and the other wells provides negative values. Despite the fact that in well MP2 nearly the same concentration of Fe(II) was detected as in well MP4, the δ^{56}Fe value is near zero like in well MP1, which is free of contamination. The first entry of contaminants in groundwater flow direction at the site is located in the vicinity of MP2. In this sector (at the former fuel storehouse), aerobic and anaerobic bacteria start to biodegrade the soluble organic compounds. Possibly, a groundwater flow of some meters is required for the equilibrium between the individual redox reactions in the system. The similar values for the iron isotopic composition of the groundwater from MP1 and MP2 are contrasting the already elevated iron content in MP2 compared to MP1. The reason for the delay of the isotopic signal with respect to the groundwater flow direction is not yet understood. Butler et al. (2005) have carried out experiments of Fe isotope fractionation during precipitation of FeS from excess aqueous Fe(II) solutions by addition of sodium sulfide solution. These studies have shown a significant kinetic isotope effect. In their experiments the FeS product was isotopically lighter compared to its aqueous counterpart, measured directly after precipitation. But due to equilibrium fractionation, during aging of FeS precipitate in contact with the residual aqueous Fe(II) in solution, the δ^{56}Fe of the solid phase increases with time and δ^{56}Fe of aqueous Fe(II) decreases with time. These isotopic fractionation is based on non-redox processes.

Such processes can affect the iron isotope ratios along the way that in the area of MP2 the Fe in the groundwater was isotopically heavier compared to the samples from the wells located further downstream. At well MP2 the degradation process of BTEX starts and the content of Fe(II) in groundwater increases. As can be seen in figure 20, the δ^{56}Fe ratios in groundwater from wells which are located downstream of the source area are up to 0.20‰ isotopically lighter than the groundwater of the uncontaminated well. Conversely, the sediment samples are isotopically heavier and, as shown in figure 20, apart from one outlier all the data points are above zero. Concerning the outlier in B3 it is suspected that due to a small-scale heterogeneity in isotopic composition, which is not clear in detail, the sediment samples from the

capillary fringe also show negative values of δ^{56}Fe.

Processing of iron by iron-reducing bacteria involves a number of steps that could fractionate iron isotopes. These include dissolution of the Fe(III) substrate, transport of dissolved Fe(III) to the cell, binding of Fe(III) at the site of reduction, and Fe reduction and release of Fe(II) (Beard et al., 2003). Despite extensive interest in the subject (e.g. Cozzarelli et al., 1994; Beard et al., 1999; Brantley et al., 2001, 2004; Jahn et al., 2005; Wiederhold et al., 2006), the mechanism for bacterial iron reduction is not well understood. Experiments by Beard et al. (1999) show an isotope fractionation mediated by dissimilatory iron reducing bacteria. In these experiments the δ^{56}Fe-values of ferrous iron in solution were up to 1.3‰ isotopically lighter than that in the substrate.

Furthermore Lovley et al. (1994b) have shown, that the bioavailability of Fe(III) from insoluble Fe(III) oxides in the aquifer increases dramatically by adding organic ligands. Schmitt et al. (1996) assumed that organic acids produced during anaerobic biological mediated degradation of BTEX might be suitable complexation agents. Alkylated aromatic acids, e.g. methylbenzoic acid isomers, benzylsuccinic acid and methylbenzylsuccinic acid isomers are identified as intermediates or metabolic dead-end products of anaerobic metabolism in fuel contaminated aquifers (Cozzarelli et al., 1994; Beller, 1995, 2002; Martus & Püttmann, 2003). These acids can act as organic ligands and might mobilise insoluble Fe(III) in the aquifer. Consequently the agents are capable of complexation of Fe(III) complexes are available for microbial iron reduction in the aquifer and might support Fe(III) reduction and consequently increasing Fe(II) concentrations in the groundwater. Brantley et al. (2004) and Wiederhold et al. (2006) have also observed a significant iron isotope fractionation by ligand-controlled mineral dissolution.

Figure 8 in chapter 4 on page 52 shows high concentrations of BTEX in the sediment at a depth of 3 to 6 m. However, in both drillings, heavier isotopic compositions are only detected at a depth of three meters. This layer is located in the unsaturated zone. In the saturated zone and the capillary fringe biological degradation of hydrocarbons occurs under anaerobic conditions. However, in the unsaturated zone suitable conditions for Fe(II) oxidization and aerobic degradation due to oxygen being constantly added by seepage water and due to seasonal variation of groundwater levels prevail.

As shown in Hettwer et al. (2006) it is very difficult to detect ferrous iron in seepage water from field sites. Therefore, little information exists concerning the reduction of Fe(III) in the unsaturated zone. As reduced Fe is isotopically lighter than the Fe(III) which remains in sediment, it is possible that an amount of the reduced Fe, which was generated in the sediment of the unsaturated zone, was then transported by seepage water to a deeper layer of the aquifer. Thereby the upper layers of the aquifer become isotopically heavier, while in the deeper sections this shift has not been ascertained. Nevertheless a distinct iron isotope fractionation between the sediment samples and the groundwater occurred. The δ^{56}Fe of ferrous iron in groundwater from the contaminated observation wells MP4, B8 and MP5 (upper section of MP-wells) is lower than in all sediment samples. The highest observed δ^{56}Fe difference between groundwater and sediment is 0.46‰. This value is much smaller than in abovementioned laboratory experiments. One reason for the observed difference between nature and experiments might be that much of the reduced Fe remains in the sediment through precipitation as secondary minerals. This is converse to laboratory experiments where it is possible to continuously separate the reduced iron in solution from the substrate.

6.7 Conclusions

The results from three years of investigations at the study site Schäferhof-Süd have shown that natural attenuation processes under anaerobic conditions are active in groundwater resulting in removal of BTEX. In the studied part of the plume the depletion of dissolved oxygen, nitrate and sulphate and the production of the biodegradation by-product Fe(II) could be detected. The observed Fe isotopic fractionation between groundwater and sediment samples in this study is assumed to be caused by different processes. According to the experiments, discussed above, the isotopic fractionation of iron could be explained by the following processes:

1. The activity of iron reducing bacteria: On the base of the study by Beard et al. (1999) fractionation of Fe isotopes can be presumed in areas with an increased content of hydrocarbons and subsequent high biological activity. The parallel reduction of sulphate and Fe(III) at the study site is observed and thus the precipitation of iron sulphide minerals can be assumed. This precipitation of

immobile iron sulphide minerals would affect the iron isotope ratios in such a way that in the area of MP2 the δ^{56}Fe values in groundwater samples was isotopically heavier compared to the other contaminated samples.

2. The formation of organic ligands through the metabolization of hydrocarbons or from natural sources: Aromatic acids generated by biodegradation of aromatic hydrocarbons can act as organic ligands and might mobilize insoluble Fe(III) in the aquifer. Such Fe(III) complexes might be available for microbial iron reduction resulting in an increase of the Fe(II) concentration in groundwater. Based on this assumption, the observed increase of Fe(II) concentrations in groundwater and the iron isotope fractionation can also be explained by the occurrence of organic ligands in the groundwater.

Results from this research show evidence of isotope fractionation due to biological degradation of hydrocarbons in the subsurface at a contaminated site, but clearly more work is needed in order to understand iron isotope fractionation mechanisms at contaminated sites and to make the analysis of iron isotopes useful for the monitoring of biodegradation at contaminated sites.

7 General conclusions and future implications

By use of the presented methods, active biological metabolism of organic material by microorganisms can be proved for the former military site Schäferhof-Süd. In the course of the coorperative project between the Umweltbundesamt, the Johann Wolfgang Goethe-University, the University of Bremen (Zentrum für Umweltforschung und Umwelttechnologie) and the alphacon GmbH, complementary studies have provided fundamental insights on, for example, the potentials and limitations of natural degradation and sorption processes of petroleum hydrocarbons and BTEX contaminants in the vadose zone (Hettwer, 2006) or the use of the detection of groundwater level fluctuations in a long term monitoring at a contaminated site (Gaab et al., 2007). A comprehensive description about the results of the processes in the subsurface of the UST Schäferhof-Süd is presented by Hettwer et al. (2006).

The BTEX contamination in the subsurface at the study site shows an unexpected spatial distribution. The contamination increases in groundwater flow direction due to additional sources of BTEX located in the downstream gradient area.

In the present study it has been shown that temporally increasing BTEX concentrations in groundwater are mainly caused by groundwater level fluctuations. The very dry summer in 2003 resulted in a drop of the groundwater level of up to 1.7 m and in a concomitant increase of BTEX concentrations from 240 µg/l to 1300 µg/l. The investigations clearly show that the groundwater level fluctuations have by far a stronger influence on the BTEX concentrations in groundwater than the influence of biological degradation. Because of that the author suggests to include the influence of groundwater level fluctuations as a parameter in modelling calculations in order to determine degradation rates. Additionally it has become evident that a long term monitoring is essential for the clarification of expected contaminant concentrations and variations in groundwater level. Hence, an evaluation of short-term increased contaminations in groundwater is much better possible.

But for the complete clarification of the interaction between groundwater level and BTEX concentrations in groundwater and the detailed understanding of the biochemical processes at the UST Schäferhof-Süd more informations about the additional BTEX-sources in the unsaturated zone are needed.

Parallel to increased BTEX concentrations, changes in redox sensitive hydrochemical parameters were likewise measured in the groundwater at the site. A simultaneous depletion of oxygen, nitrate-, iron- and sulphate reduction and methanogenese was evident. Organic acids were identified as metabolic by-products of biodegradation as well. Benzoic acid, C_1-, C_2- and C_3-benzoic acid were determined in all contaminated wells with considerable concentrations. Thereby clearly indicating active biological metabolism of organic material by microorganisms at the location UST Schäferhof-Süd.

But increased concentrations of metabolites in groundwater can also show the inhibition of biodegradation. If the biodegradation pathway is interrupted or uncompleted mostly caused by toxic effects or a lack of electron acceptors, an increase of metabolites can be observed (McKelvie et al., 2005). Hence, higher metabolite concentrations can be found in the contaminant source compared to the fringe of a plume where the supply of electron acceptors is higher. At the site Schäferhof-Süd no samples from the fringe of the plume could be analysed due to the allocation of groundwater wells. Hence, a verification of the connection from BTEX concentrations and metabolite concentrations in groundwater was not possible. But this would be of interest for a detailed unterstanding of the biological metabolism of organic material by microorganisms.

Biodegradation of BTEX include the metabolic reduction of iron that can produce a measurable Fe isotopic fractionation in sediment and groundwater. Experiments have proved biological fractionation of Fe isotopic composition through Fe reducing bacteria which are able to use the abundant Fe(III) in minerals as an energy source (Beard et al., 1999, 2003).

For further hydrogeochemical testing of groundwater samples, it would be helpful to have a second background well in the uncontaminated zone and two more wells downstream of the source ares. For the interpretation and evaluation of the results a clear chemical classification of groundwater is necessary. Only one well can not represent the variation of hydrogeochemical data in the uncontaminated zone.

Especially for further studies in the research area of isotope geochemistry it is necessary to analyse more sediment and groundwater samples. The observed significant iron isotope fractionation between groundwater and sediment samples in this study show evidence of isotope fractionation due to biological degradation of

hydrocarbons in the subsurface at a contaminated site. These data are a good basis for a further research project to unterstand the fractionation mechanisms at the site. The follow points should additionally attract interest in further investigations.

Due to new cognitions for the handling of groundwater samples filtration and acidification is recommended during sampling. Filtration of the sample will assure that no particles from sediment will be analysed together with the water sample.

It is well known that the oxidation of ferrous iron at neutral pH, like in the groundwater of Schäferhof-Süd, proceeds very fast and precipitation of secondary Fe(III) hydroxide phases occurs within minutes. Acidification would avoid this oxidation process and ensure that all Fe(II) remains in solution.

In order to interpret both, iron concentration and iron isotope data, it would be good to know which Fe solid phases are present in the sampled material. Informations on the Fe mineralogy of the material could give informations about the potential bioavailability of Fe(III) from the aquifer sediment material which could be used as electron acceptor by iron-reducing bacteria. The detailed identification of minor Fe-containing mineralogical phases in sedimentary material should be included in future investigations.

In the context of NA it could be shown that the analysis of electron acceptors, metabolic by-products and iron isotopes are helpful tools to provide evidence for intrinsic biodegradation processes in groundwater at contaminated sites.

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9 Appendix

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Table 8: Groundwater level [m ASL] UST Schäferhof–Süd

	Jan 02 [m ASL]	Mrz 02 [m ASL]	Jun 02 [m ASL]	Sep 02 [m ASL]	Dec 02 [m ASL]	Mrz 03 [m ASL]	Jun 03 [m ASL]	Sep 03 [m ASL]	Dec 03 [m ASL]	Mrz 04 [m ASL]	Jun 04 [m ASL]	Aug 04 [m ASL]
B 8	23,91		24,52		24,43	25,14	24,46					
MP1f	24	24,74	24,82	24,42	24,5	25,22	24,7	23,82	23,57	24,08	24,1	23,82
MP2f	23,95	24,90	24,79	24,46	24,16	25,2	24,65	23,96	23,52	24,04	24,06	23,77
MP3f	23,96	24,89	24,72	24,23	24,46	25,2	24,64	23,97	23,52	24,04	24,06	23,77
MP4f	23,95	24,85	24,76	24,46	24,47	25,18	24,54	23,95	23,36	24,02	24,04	23,75
MP5f	23,88	24,74	24,25	24,10	24,40	25,12	24,59	23,47	23,47	23,98	24,01	24,06

Table 9: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 07.01.2002

sample		B 8	MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	6	< 1	< 1	< 1	< 1	< 1	9	< 1	2	41	33
Toluene	µg/l	10	< 1	< 1	< 1	< 1	< 1	< 1	< 1	71	130	370
Ethylbenzene	µg/l	20	< 1	< 1	< 1	7	< 1	21	< 1	52	110	190
m/p-xylene	µg/l	76	< 1	< 1	< 1	43	< 1	120	< 1	340	390	770
o-xylene	µg/l	11	< 1	< 1	< 1	< 1	< 1	1	< 1	76	65	140
Sum BTEX	µg/l	120				50		150		540	740	1.500
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	3	3	6
Cumene	µg/l	2	< 1	< 1	< 1	< 1	< 1	3	< 1	4	6	13
Mesitylene	µg/l	3	< 1	< 1	< 1	4	< 1	7	< 1	8	15	60
Pseudocumene	µg/l	10	< 1	< 1	< 1	15	< 1	19	< 1	21	53	160
Hemellitol	µg/l	4	< 1	< 1	< 1	7	< 1	7	< 1	7	13	44
Sum aromatics	µg/l	19				26		36		43	90	280
Benzoic acid	mg/l	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01
Index of hydrocarbon	mg/l	0,2	< 0,1	< 0,1	< 0,1	0,2	< 0,1	0,1	< 0,1	0,1	0,1	0,2
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	41,3	0,55	0,06	0,04	26,3	0,17	27,5	0,05	40,4	68,6	55,1
Manganese	mg/l	1,97	0,06	< 0,01	0,04	1,18	0,10	2,19	0,05	0,83	3,59	2,07
Nitrate	mg/l	< 1	75	50	64	14	67	10	30	14	< 1	2
Phospate	mg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Sulphate	mg/l	10	42	30	26	15	35	21	26	15	2	22
TOC	mg/l	23	7	7	4	18	3	25	3	29	21	30
DOC	mg/l	23	5	6	4	17	3	25	3	26	20	22
Acid capacity KS 4,3	mmol/l	2,24	0,08	0,10	0,11	2,54	0,18	2,84	0,55	3,44	4,42	3,32
Ammonium	mg/l	1,24	< 0,03	< 0,03	< 0,03	0,53	< 0,03	0,29	< 0,03	0,94	1,43	2,04
Hydrogen carbonate	mmol/l	2,24	0,08	0,10	0,11	2,54	0,18	2,84	0,55	3,44	4,42	3,32
Oxygen	mg/l	0,13	0,14	4,59	0,67	0,48	1,03	0,2	0,57	0,12	0,1	0,16
Conductivity	µS/cm	301	349	247	275	316	291	325	217	357	456	388
Eh	mV	+100	+490	+480	+430	+110	+340	+140	+350	+100	+80	+140
Temperature	℃	11,8	11,2	11,0	11,7	11,9	11,8	11,8	11,8	11,8	11,4	11,2
pH	-	6,1	5,6	5,8	5,9	6,3	6	6,2	6,3	6,3	6,6	6,4

Table 10: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 05.03.2002

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MPf	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	< 1	< 1	< 1	3	< 1	6	< 1	4	120	95
Toluene	µg/l	< 1	< 1	< 1	1	< 1	2	< 1	120	430	760
Ethylbenzene	µg/l	< 1	< 1	< 1	26	< 1	34	< 1	150	330	300
m/p-Xylene	µg/l	< 1	1	1	94	3	110	2	530	820	760
o-Xylene	µg/l	< 1	< 1	< 1	3	< 1	3	< 1	190	300	330
Sum BTEX	µg/l		1	1	130	3	160	2	990	2.000	2.200
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Cumene	µg/l	< 1	< 1	< 1	3	< 1	5	< 1	15	24	22
Mesitylene	µg/l	< 1	< 1	< 1	8	< 1	11	< 1	34	48	46
Pseudocumene	µg/l	< 1	1	1	24	2	32	2	100	140	130
Hemellitol	µg/l	< 1	< 1	< 1	14	2	15	< 1	44	67	65
Sum aromatics	µg/l		1	1	49	4	63	2	190	280	260
Benzoic acid	mg/l	< 0,01	< 0,01		0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,3	< 0,1	0,5	< 0,1	0,5	1,0	0,7
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	< 0,02	< 0,02	< 0,02	25,5	< 0,02	8,91	0,03	27,2	73,3	76,2
Manganese	mg/l	0,03	< 0,01	0,02	1,44	0,05	0,78	0,01	0,61	3,75	3,55
Nitrate	mg/l	72	62	75	15	58	14	42	3	< 1	< 1
Phospate	mg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Sulphate	mg/l	42	29	32	15	27	25	24	12	1	9
TOC	mg/l	2	3	2	28	2	9	2	20	20	18
DOC	mg/l	2	3	2	26	2	9	2	20	20	17
Acid capacity KS 4,3	mmol/l	0,28	0,38	0,34	2,45	0,45	1,81	0,47	2,50	4,50	4,34
Ammonium	mg/l	< 0,03	< 0,03	< 0,03	0,73	< 0,03	0,23	< 0,03	0,91	1,58	1,67
Hydrogen carbonate	mmol/l	0,28	0,38	0,34	2,45	0,45	1,81	0,47	2,50	4,50	4,34
Oxygen	mg/l	0,34	3,37	0,35	3,5	5,46	3,64	0,58	0,22	0,2	0,21
Conductivity	µS/cm	379	299	363	329	289	295	236	311	498	490
Eh	mV	450	540	430	160	400	190	400	160	100	110
Temperature	℃	11,2	10,5	11,1	10,5	11,4	10,6	11,3	10,6	11,1	10,2
pH	-	4,8	6	5,9	6,4	6,1	6,5	6,2	6,5	6,7	6,6

Table 11: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 03.06.2002

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f	B 8
Benzene	µg/l	< 1	< 1	< 1	7	< 1	41	< 1	14	130	90	26
Toluene	µg/l	< 1	< 1	< 1	1	< 1	8	< 1	360	380	1.200	92
Ethylbenzene	µg/l	< 1	< 1	1	2	< 1	110	< 1	440	480	500	140
m/p-Xylene	µg/l	< 1	< 1	1	34	< 1	190	1	850	560	750	130
o-Xylene	µg/l	< 1	< 1	2	4	< 1	11	< 1	570	240	470	100
Sum BTEX	µg/l			4	48		360	1	2.200	1.800	3.000	490
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	1	1	4	< 1
Cumene	µg/l	< 1	< 1	< 1	< 1	< 1	11	< 1	36	43	45	14
Mesitylene	µg/l	< 1	< 1	< 1	12	< 1	38	< 1	96	86	110	28
Pseudocumene	µg/l	< 1	< 1	< 1	6	< 1	69	1	270	250	290	82
Hemellitol	µg/l	< 1	< 1	< 1	10	< 1	47	< 1	120	94	120	34
Sum aromatics	µg/l				28		170	1	520	470	570	160
Benzoic acid	mg/l	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,5	< 0,1	0,4	0,1	0,3	0,6	0,3	0,1
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	< 0,02	0,11	0,06	35,1	< 0,02	43,0	< 0,02	29,7	35,9	5,26	202
Manganese	mg/l	0,03	0,02	0,02	5,68	0,08	2,15	< 0,01	0,91	3,17	1,46	1,96
Nitrate	mg/l	65	46	78	2	52	8	33	1	< 1	1	< 1
Phospate	mg/l	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Sulphate	mg/l	45	31	39	20	27	44	25	10	4	28	13
Methane	µg/l	<0,02	<0,02	<0,02	1200	<0,02	4100	<0,02	8500	7100	2100	3400
TOC	mg/l	4	5	4	65	5	35	3	47	24	27	21
DOC	mg/l	4	4	3	53	4	34	3	41	20	25	19
Acid capacity KS 4,3	mmol/l	0,38	0,49	0,37	3,59	0,51	2,75	0,51	3,30	3,45	2,98	1,63
Ammonium	mg/l	< 0,03	< 0,03	< 0,03	1,57	< 0,03	0,71	< 0,03	2,07	2,10	2,10	1,41
Hydrogen carbonate	mmol/l	0,38	0,49	0,37	3,59	0,51	2,75	0,51	3,30	3,45	2,98	1,63
Oxygen	mg/l	0,42	5,49	0,22	0,24	0,67	0,24	1,00	0,39	0,40	0,68	0,31
Conductivity	µS/cm	358	257	382	605	272	467	213	450	464	474	344
Eh	mV	444	426	376	89	390	130	233	123	81	109	97
Temperature	℃	10,8	10,4	11,0	10,8	10,9	10,9	11,0	10,8	10,8	10,8	11,0
pH	-	5,7	6,0	5,7	6,4	6,1	6,2	6,1	6,3	6,5	6,4	6,3

Table 12: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 11.09.2002

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	< 1	< 1	< 1	15	2	47	< 1	6	120	39
Toluene	µg/l	< 1	< 1	< 1	5	4	10	2	180	370	330
Ethylbenzene	µg/l	< 1	< 1	< 1	65	4	150	5	460	440	290
m/p-Xylene	µg/l	< 1	< 1	1	300	11	540	14	1.500	1.100	890
o-Xylene	µg/l	< 1	< 1	< 1	12	5	16	7	510	320	310
Sum BTEX	µg/l			1	400	26	760	28	2.700	2.400	1.900
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	2	1
Cumene	µg/l	< 1	< 1	< 1	4	5	14	3	55	44	37
Mesitylene	µg/l	< 1	< 1	< 1	48	1	53	2	130	96	110
Pseudocumene	µg/l	< 1	< 1	< 1	100	7	150	6	330	300	280
Hemellitol	µg/l	< 1	< 1	< 1	69	5	71	2	130	110	110
Sum aromatics	µg/l				220	18	290	13	650	550	540
Benzoic acid	mg/l	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,3	< 0,1	0,3	< 0,1	0,7	0,3	0,4
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	0,17	1,08	0,10	64,5	0,07	55,5	0,53	48,7	76,3	30,2
Nitrate	mg/l	65	24	56	< 1	25	2	22	< 1	< 1	< 1
Sulphate	mg/l	37	22	33	21	41	25	34	9	2	15
TOC	mg/l	5	6	5	49	12	41	6	55	25	30
DOC	mg/l	4	5	4	46	11	39	5	53	25	26
Acid capacity KS 4,3	mmol/l	0,56	0,78	0,66	3,18	1,32	3,66	0,84	3,78	3,70	4,48
Hydrogen carbonate	mmol/l	0,56	0,78	0,66	3,18	1,32	3,66	0,84	3,78	3,70	4,48
Conductivity	µS/cm	319	178	283	520	278	441	222	493	469	508
pH	-	5,8	6,2	6,0	6,5	6,2	6,4	6,3	6,5	6,8	6,7
Eh	mV	+430	+410	+370	+30	+230	+100	+210	+100	+60	+80
Oxygen	mg/l	5,0	8,4	4,3	2,5	3,1	2,3	3,8	2,2	2,4	1,9
Temperature	℃	11,0	12,0	11,5	13,3	12,5	13,6	12,0	13,3	11,9	14,1

Table 13: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 11.09.2002

sample		B8	MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	32	< 1	< 1	< 1	16	2	53	< 1	10	180	23
Toluene	µg/l	88	< 1	< 1	< 1	7	< 1	12	< 1	320	470	250
Ethylbenzene	µg/l	230	< 1	< 1	< 1	220	4	180	2	650	590	230
m/p-Xylene	µg/l	450	< 1	< 1	< 1	590	11	530	6	1.900	1.500	840
o-Xylene	µg/l	140	< 1	< 1	< 1	15	< 1	14	< 1	640	390	270
Sum BTEX	µg/l	940				850	17	790	8	3.500	3.100	1.600
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	2	2	2
Cumene	µg/l	25	< 1	< 1	< 1	19	1	16	< 1	56	49	36
Mesitylene	µg/l	48	< 1	< 1	< 1	59	1	46	1	160	110	94
Pseudocumene	µg/l	130	< 1	< 1	< 1	130	3	130	2	400	330	260
Hemellitol	µg/l	56	< 1	< 1	< 1	72	2	66	1	170	130	120
Sum aromatics	µg/l	260				280	7	260	4	790	620	510
Benzoic acid	mg/l	0,05	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	0,02	< 0,01	< 0,01	0,02	< 0,01
Index of hydrocarbon	mg/l	1,0	< 0,1	< 0,1	< 0,1	0,7	< 0,1	0,8	< 0,1	0,7	0,8	0,5
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	42,5	0,06	0,04	< 0,02	32,0	0,03	37,7	0,07	50,6	74,4	12,8
Manganese	mg/l	1,78	0,01	< 0,01	< 0,01	1,94	0,05	2,48	< 0,01	1,14	4,08	0,64
Nitrate	mg/l	1	68	49	57	8	30	3	42	< 1	< 1	5
Sulphate	mg/l	11	34	34	42	10	31	19	40	5	< 1	14
Methane	µg/l	51	<10	<10	<10	1300	<10	4100	<10	7300	6900	1100
TOC	mg/l	33	5,2	4,8	4,2	28	7	42	3,7	75	31	27
DOC	mg/l	23	3,8	4,6	3,5	24	7	38	3,4	73	30	22
Acid capacity KS 4,3	mmol/l	0,94	0,60	0,45	0,37	2,00	1,13	3,16	0,47	3,06	2,39	3,29
Ammonium	mg/l	1,61	0,08	0,09	0,07	1,50	0,09	1,98	0,13	3,64	1,95	1,49
Hydrogen carbonate	mmol/l	0,94	0,60	0,45	0,37	2,00	1,13	3,16	0,47	3,06	2,39	3,29
Oxygen	mg/l	0,3	0,3	6,0	0,3	0,1	0,4	4,9	2,8	0,3	0,2	0,5
Conductivity	µS/cm	369	328	237	308	350	253	485	223	475	481	415
Eh	mV	+120	+490	+450	+420	+80	+170	+120	+240	+80	+80	+150
Temperature	°C	13,0	11,8	12,1	12,1	12,8	12,1	13,0	12,2	13,0	12,6	12,9
pH	-	6,3	5,8	6,0	5,9	6,4	6,2	6,4	6,1	6,3	6,6	6,5

Table 14: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 12.03.2003

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	< 1	< 1	< 1	10	2	49	< 1	8	160	29
Toluene	µg/l	1	< 1	< 1	3	< 1	10	< 1	250	600	490
Ethylbenzene	µg/l	< 1	< 1	1	8	1	180	2	370	770	390
m/p-Xylene	µg/l	< 1	< 1	3	210	2	570	4	1.500	2.200	1.400
o-Xylene	µg/l	< 1	< 1	< 1	13	< 1	13	< 1	540	740	360
Sum BTEX	µg/l	1		4	240	5	820	6	2.700	4.500	2.700
Styrene	µg/l	< 1	< 1	< 1	1	< 1	1	< 1	16	24	12
Cumene	µg/l	< 1	< 1	< 1	< 1	2	15	< 1	36	67	38
Mesitylene	µg/l	< 1	< 1	< 1	40	< 1	56	< 1	120	230	140
Pseudocumene	µg/l	< 1	< 1	1	54	2	170	1	330	650	360
Hemellitol	µg/l	< 1	< 1	< 1	61	< 1	73	< 1	130	260	140
Sum aromatics	µg/l			1	160	4	320	1	630	1.200	690
Benzoic acid	mg/l	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,3	< 0,1	< 0,1	0	0,4	0,7	0,3
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	< 0,02	< 0,02	0,05	79	0,05	37	0,07	39	80	20
Nitrate	mg/l	60	43	59	< 1	39	1	30	< 1	< 1	< 1
Sulphate	mg/l	42	33	47	31	32	41	35	4,2	6	23
TOC	mg/l	3,1	4,1	2,5	76	6,2	39	3,1	45	35	38
DOC	mg/l	1,8	3,6	2,5	67	3,6	32	1,5	36	33	35
Acid capacity KS 4,3	mmol/l	0,33	0,57	0,35	2,75	1,04	2,53	0,48	2,11	2,66	5,52
Hydrogen carbonate	mmol/l	0,33	0,57	0,35	2,75	1,04	2,53	0,48	2,11	2,66	5,52
Oxygen	mg/l	0,1	5,3	0,3	0,3	0,3	0,2	1,9	0,2	0,2	0,3
Conductivity	µS/cm	336	256	359	499	305	451	265	355	482	634
Eh	mV	+460	+430	+200	+60	+330	+130	+370	+90	+100	+110
Temperature	℃	11,4	10,3	11,1	9,4	11,1	10,1	11,3	10,2	10,9	9,2
pH	-	5,6	5,5	5,4	5,9	5,8	5,9	5,6	6	6,1	6,2

Table 15: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 03.06.2003

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f	B8	B8
Benzene	µg/l	< 1	< 1	< 1	28	< 1	86	< 1	11	230	15	60	45
Toluene	µg/l	< 1	< 1	< 1	11	< 1	14	< 1	420	600	300	320	320
Ethylbenzene	µg/l	< 1	< 1	< 1	160	< 1	300	< 1	1.100	860	500	530	430
m/p-Xylene	µg/l	< 1	< 1	2	520	2	950	1	4.100	2.200	1.800	1.500	1.200
o-Xylene	µg/l	< 1	< 1	< 1	22	< 1	23	< 1	1.500	660	410	440	440
Sum BTEX	µg/l			2	740	2	1.400	1	7.100	4.600	3.000	2.900	2.400
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	2	4	1	< 1	1
Cumene	µg/l	< 1	< 1	< 1	11	< 1	25	< 1	87	76	60	48	41
Mesitylene	µg/l	< 1	< 1	< 1	68	< 1	85	< 1	250	180	190	130	110
Pseudocumene	µg/l	< 1	< 1	< 1	170	1	250	< 1	690	530	460	400	320
Hemellitol	µg/l	< 1	< 1	< 1	95	1	120	< 1	310	210	200	160	130
Sum aromatics	µg/l				340	2	480		1.300	1.000	910	740	600
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,5	< 0,1	0,8	< 0,1	1	0,8	0,5	1,2	0,6
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	0,02	0,04	0,04	64	< 0,02	35	0,16	31	52	5,8	41	37
Nitrate	mg/l	55,2	29	56,1	0,13	31,3	0,12	18,9	0,17	0,1	0,14	0,13	0,1
Sulphate	mg/l	33,2	23,5	33,7	58,1	26,1	28,4	19,2	2,6	1,9	14,2	1,7	4,5
Methane	µg/l	26	<10	<10	1500	26	6200	27	6200	6800	1500	4900	6800
TOC	mg/l	5	5,5	3,6	76	4,4	35	2,6	53	29	51	50	50
DOC	mg/l	4,7	5,3	3,4	65	4,3	25	2,4	44	21	42	43	40
Acid capacity KS 4,3	mmol/l	0,32	0,59	0,33	3,99	0,96	3,06	0,62	3,86	4,43	7,50	4,37	3,59
Hydrogen carbonate	mmol/l	0,32	0,59	0,33	3,99	0,96	3,06	0,62	3,86	4,43	7,50	4,37	3,59
Oxygen	mg/l	0,2	6,9	0,2	0,1	0,1	0,4	5,9	0,1	0,6	0,4	0,2	0,1
Conductivity	µS/cm	293	229	342	568	278	395	169	417	493	749	471	409
Eh	mV	470	440	410	160	290	180	320	130	90	120	130	130
Temperature	℃	10,7	10,1	10,7	10,6	10,9	10,6	11,1	10,5	10,7	10,3	10,8	10,9
pH	-	6,1	6,1	5,8	6,3	6,3	6,1	6,2	6,4	6,6	6,7	6,4	6,4
												sampling depth 5m	sampling depth 9m

Table 16: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 04.09.2003

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	< 1	< 1	< 1	23	< 1	76	< 1	9	160	38
Toluene	µg/l	< 1	< 1	< 1	11	< 1	18	< 1	340	410	530
Ethylbenzene	µg/l	< 1	< 1	< 1	250	3	270	4	790	610	420
m/p-Xylene	µg/l	< 1	< 1	< 1	1000	7	890	9	3200	1500	1600
o-Xylene	µg/l	< 1	< 1	< 1	18	1	20	1	980	460	460
Sum BTEX	µg/l				1300	11	1300	14	5300	3100	3000
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	4	3
Cumene	µg/l	< 1	< 1	< 1	19	2	22	< 1	59	52	45
Mesitylene	µg/l	< 1	< 1	< 1	80	2	70	2	210	120	180
Pseudocumene	µg/l	< 1	< 1	< 1	230	3	220	3	530	370	440
Hemellitol	µg/l	< 1	< 1	< 1	100	2	100	2	230	140	180
Sum aromatics	µg/l				430	9	410	7	1.000	690	850
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,4	< 0,1	0,7	< 0,1	0,5	0,7	0,2
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	< 0,02	0,02	< 0,02	28	< 0,02	37	0,13	51	71	24
Nitrate	mg/l	54,3	31,2	51,6	0,15	30,4	0,13	20,1	0,15	0,12	0,13
Sulphate	mg/l	33,8	25,7	42,3	48,6	27,5	31,6	25,8	3,2	2,1	18,9
TOC	mg/l	3,8	3,4	3,3	22	5,6	25	3,4	38	23	23
DOC	mg/l	3,5	2,8	3,1	21	4,8	3	3,4	37	23	22
Acid capacity KS 4,3	mmol/l	0,3	0,48	0,44	2,39	1,45	2,93	0,65	4,23	4,5	3,89
Hydrogen carbonate	mmol/l	0,3	0,48	0,44	2,39	1,45	2,93	0,65	4,23	4,5	3,89
Oxygen	mg/l	0,1	7,2	2,9	0,1	0,7	0,1	6,3	0,3	0,3	0,3
Conductivity	µS/cm	310	219	213	298	230	354	160	454	450	399
Eh	mV	+420	+430	+430	+50	+290	+90	+310	+80	+80	+90
Temperature	℃	10,7	11,1	11,4	13,0	11,5	13,2	11,5	13,3	11,5	13,1
pH	-	6,0	6,3	6,2	6,8	6,7	6,6	6,3	6,7	6,9	6,9

Table 17: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 04.12.2003

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	< 1	< 1	< 1	12	< 1	37	< 1	6	130	33
Toluene	µg/l	< 1	< 1	< 1	5	< 1	11	< 1	230	280	340
Ethylbenzene	µg/l	< 1	< 1	< 1	170	< 1	170	2	540	440	320
m/p-Xylene	µg/l	< 1	< 1	1	600	1	500	2	1.800	1.100	910
o-Xylene	µg/l	< 1	< 1	< 1	13	< 1	10	< 1	530	310	290
Sum BTEX	µg/l			1	800	1	730	4	3.100	2.300	1.900
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	17	12	11
Cumene	µg/l	< 1	< 1	< 1	17	< 1	18	< 1	48	38	37
Mesitylene	µg/l	< 1	< 1	< 1	60	< 1	39	< 1	140	93	110
Pseudocumene	µg/l	< 1	< 1	< 1	170	< 1	120	1	360	270	290
Hemellitol	µg/l	< 1	< 1	< 1	73	< 1	54	< 1	160	110	120
Sum aromatics	µg/l				320		230	1	730	520	570
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	1,2	< 0,1	1	< 0,1	1,5	0,8	0,7
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	0,09	0,21	0,06	29	0,2	31	0,32	53	88	35
Nitrate	mg/l	62,6	40,9	40,3	1,2	9,3	4,4	24,1	0,17	0,07	0,16
Sulphate	mg/l	37,7	26,8	22,7	14,3	20,6	8,4	21,2	6,6	0,29	10,7
Methane	µg/l	<10	<10	<10	1200	23	6800	12	9000	7300	2000
TOC	mg/l	7,1	7,8	6,9	19	9	25	9	18	9,1	7,4
DOC	mg/l	5,3	7,7	5,6	17	8,7	16	5	18	9	6,5
Acid capacity KS 4,3	mmol/l	0,32	0,42	0,42	1,6	1,20	2,4	0,66	2,60	3,5	2,10
Hydrogen carbonate	mmol/l	0,32	0,42	0,42	1,6	1,20	2,4	0,66	2,60	3,5	2,10
Oxygen	mg/l	0,2	4,3	3	0,2	2,4	0,5	3,8	0,2	0,3	0,6
Conductivity	µS/cm	313	237	234	275	217	340	204	430	496	308
Eh	mV	+460	+460	+430	+150	+270	+170	+270	+150	+100	+120
Temperature	℃	11,1	11,5	11,7	12,4	11,8	12,6	11,9	12,7	11,9	12,3
pH	-	5,8	5,9	5,8	6,4	6,3	6,3	6,1	6,4	6,6	6,5

Table 18: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 17.03.2004

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	< 1	< 1	< 1	9	< 1	45	< 1	5	190	74
Toluene	µg/l	< 1	< 1	< 1	7	< 1	11	1	120	590	1100
Ethylbenzene	µg/l	< 1	< 1	< 1	120	< 1	88	2	68	990	990
m/p-Xylene	µg/l	< 1	< 1	< 1	490	2	480	5	1.100	2.800	3.400
o-Xylene	µg/l	< 1	< 1	< 1	21	< 1	13	1	330	710	960
Sum BTEX	µg/l				650	2	640	9	1.600	5.300	6.500
Styrene	µg/l	< 1	< 1	< 1	< 1	< 1	< 1	< 1	12	25	34
Cumene	µg/l	< 1	< 1	< 1	10	2	9	< 1	9	89	100
Mesitylene	µg/l	< 1	< 1	< 1	79	< 1	45	< 1	120	260	340
Pseudocumene	µg/l	< 1	< 1	< 1	120	< 1	88	1	210	750	870
Hemellitol	µg/l	< 1	< 1	< 1	110	< 1	68	< 1	130	300	370
Sum aromatics	µg/l				320	2	210	1	480	1.400	1.700
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,3	< 0,1	0,1	< 0,1	0,2	0,3	0,2
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	0,04	0,07	0,05	36	0,1	35	0,16	36	91	29
Nitrate	mg/l	65	51	66	2	23	1	27	< 1	< 1	2
Sulphate	mg/l	40	26	41	28	21	28	27	10	4	42
Methane	µg/l	<20	<20	<20	1300	38	5400	<20	5700	10000	2400
TOC	mg/l	8	7	6	31	11	34	8	26	38	34
DOC	mg/l	5	6	5	29	10	32	8	24	32	31
Acid capacity KS 4,3	mmol/l	0,34	0,54	0,36	3	1,30	3,7	0,83	2,60	4,8	5,20
Hydrogen carbonate	mmol/l	0,34	0,54	0,36	3	1,30	3,7	0,83	2,60	4,8	5,20
Oxygen	mg/l	0,2	2,9	0,1	0,1	2,4	0,2	4,5	0,1	0,1	0,3
Conductivity	µS/cm	346	265	358	357	228	449	216	281	516	581
Eh	mV	360	380	320	130	280	160	280	130	100	130
Temperature	℃	11,1	10,6	11,1	10,4	11,4	10,6	11,3	10,5	10,9	10,0
pH	-	5,9	6,1	5,8	6,6	6,4	6,5	6,2	6,5	6,6	6,7

Table 19: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 08.06.2004

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	< 1	< 1	< 1	11	< 1	88	< 1	12	220	110
Toluene	µg/l	< 1	< 1	1	6	< 1	22	< 1	370	540	1500
Ethylbenzene	µg/l	< 1	< 1	2	190	< 1	390	1	720	960	1.000
m/p-Xylene	µg/l	< 1	< 1	6	480	< 1	1200	3	2.000	2.500	3.500
o-Xylene	µg/l	< 1	< 1	< 1	16	< 1	27	< 1	570	530	810
Sum BTEX	µg/l			9	700		1.700	4	3.700	4.800	6.900
Styrene	µg/l	< 1	< 1	< 1	1	< 1	2	< 1	21	21	35
Cumene	µg/l	< 1	< 1	< 1	25	< 1	37	< 1	69	94	110
Mesitylene	µg/l	< 1	< 1	< 1	94	< 1	100	< 1	220	240	340
Pseudocumene	µg/l	< 1	< 1	1	270	< 1	340	< 1	560	700	830
Hemellitol	µg/l	< 1	< 1	1	120	< 1	160	< 1	250	290	360
Sum aromatics	µg/l			2	510		640		1.100	1.300	1.700
Index of hydrocarbon	mg/l	< 0,1	< 0,1	< 0,1	0,4	< 0,1	0,2	< 0,1	0,8	0,6	0,4
TPH > C40	mg/l	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1	< 0,1
Iron	mg/l	0,04	0,07	0,05	27	0,14	20	0,44	49	98	33
Nitrate	mg/l	70	60	73	6	44	4	15	< 1	< 1	1
Sulphate	mg/l	39	27	40	21	28	16	18	16	< 1	20
Methane	µg/l	<10	<10	<10	590	<10	5800	<10	9300	12000	4000
TOC	mg/l										
DOC	mg/l										
Acid capacity KS 4,3	mmol/l	0,45	0,68	0,57	1,33	1,12	3,17	0,93	2,53	3,57	6,30
Hydrogen carbonate	mmol/l	0,45	0,68	0,57	1,33	1,12	3,17	0,93	2,53	3,57	6,30
Oxygen	mg/l	0,2	2,8	0,1	0,2	0,3	0,5	5,3	0,1	0,1	0,3
Conductivity	µS/cm	344	293	363	287	283	423	162	380	557	724
Eh	mV	+460	+460	+380	+80	+290	+120	+330	+90	+70	+100
Temperature	℃	10,6	10,2	10,6	10,5	10,9	10,8	10,8	10,7	10,6	10,4
pH	-	5,8	6,2	6	6,6	6,6	6,6	6,5	6,7	6,8	6,9

Table 20: UST Schäferhof-Süd
Laboratory analytical results for groundwater sampling: 31.08.2004

sample		MP1t	MP1f	MP2t	MP2f	MP3t	MP3f	MP4t	MP4f	MP5t	MP5f
Benzene	µg/l	<1	<1	<1	11	<1	68	<1	10	230	110
Toluene	µg/l	<1	<1	<1	6	<1	17	<1	350	520	1000
Ethylbenzene	µg/l	<1	<1	<1	120	<1	260	1	750	680	850
m/p-Xylene	µg/l	<1	<1	<1	320	<1	870	8	2.700	1.700	2.800
o-Xylene	µg/l	<1	<1	<1	12	<1	18	9	720	370	640
Sum BTEX	µg/l				470		1.200	18	4.500	3.500	5.400
Styrene	µg/l	<1	<1	<1	<1	<1	<1	<1	24	14	25
Cumene	µg/l	<1	<1	<1	8	<1	21	<1	65	59	92
Mesitylene	µg/l	<1	<1	<1	42	<1	64	1	230	140	270
Pseudocumene	µg/l	<1	<1	<1	150	<1	220	4	540	420	700
Hemellitol	µg/l	<1	<1	<1	72	<1	100	2	240	170	300
Sum aromatics	µg/l				270		410	7	1.100	800	1.400
Index of hydrocarbon	mg/l	<0,1	<0,1	<0,1	0,7	<0,1	0,7	<0,1	1,3	1,2	1,2
TPH > C40	mg/l	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Iron	mg/l	0,11	0,11	<0,02	31	<0,02	22	0,37	60	78	36
Nitrate	mg/l	99	59	75	5	44	9	14	<1	<1	<1
Sulphate	mg/l	34	23	29	14	28	17	18	26	<1	5
Methane	µg/l	<10	<10<	<10	1500	160	6400	570	14000	15000	5200
TOC	mg/l										
DOC	mg/l										
Acid capacity KS 4,3	mmol/l	0,42	0,52	0,45	2,82	1,00	3,03	0,85	4,98	5,61	7,19
Hydrogen carbonate	mmol/l	0,42	0,52	0,45	2,82	1,00	3,03	0,85	4,98	5,61	7,19
Oxygen	mg/l	0,5	5,2	0,4	0,2	0,8	0,8	6	0,3	0,2	0,3
Conductivity	µS/cm	387	270	343	326	293	348	187	533	531	697
Eh	mV	+520	+490	+430	+30	+270	+80	+270	+40	+70	+70
Temperature	℃	10,6	10,9	11	10,5	11,1	12,4	11,2	12,7	11,2	12,2
pH	-	5,9	6,2	6	6,5	6,5	6,4	6,3	6,4	6,7	6,7

Table 21: UST Schäferhof-Süd
Laboratory analytical results for metabolites of BTEX and fatty acids: 03.06.2002

sample	B8	MP1f	MP1t	MP2f	MP2t	MP3f	MP3t	MP4f	MP4t	MP5f	MP5t
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
metabolites											
Benzoic acid	0,00	0,00	0,00	0,00	0,00	0,00	8,66	0,00	0,00	0,00	0,00
Methylbenzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Methylbenzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Methylbenzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C2-Benzoic acid (i)	0,00	0,00	0,00	1,09	0,00	0,39	0,00	0,00	0,00	0,00	0,45
C2-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C2-Benzoic acid (i)	10,50	0,00	0,00	42,44	0,00	0,00	0,00	0,00	0,00	0,00	20,79
C2-Benzoic acid (i)	1,74	0,00	0,00	38,05	0,00	43,32	0,00	0,00	0,00	0,00	0,00
C2-Benzoic acid (i)	2,55	0,00	0,00	39,95	0,00	0,00	0,00	0,00	0,00	0,00	18,69
C2-Benzoic acid (i)	0,94	0,00	0,00	9,79	0,00	10,81	0,00	0,00	0,00	0,00	9,40
C3-Benzoic acid (i)	2,49	0,00	0,00	2,87	0,00	6,34	0,00	12,24	0,00	0,00	3,90
C2-Benzoic acid (i)	4,29	0,00	0,00	6,74	0,00	3,61	0,00	3,60	0,00	0,00	5,95
C2-Benzoic acid (i)	0,00	0,00	0,00	3,07	0,00	0,00	0,00	13,07	0,00	0,00	1,59
C2-Benzoic acid (i)	9,68	0,00	0,00	50,45	0,00	2,02	0,00	3,03	0,00	0,00	8,25
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	4,35	0,00	7,10	0,00	0,78	0,00
C2-Benzoic acid (i)	0,00	0,00	0,00	8,33	0,00	6,04	0,00	2,38	0,00	0,17	0,21
C3-Benzoic acid (i)	6,36	0,00	0,00	5,10	0,00	0,00	0,00	1,68	0,00	0,93	10,76
C3-Benzoic acid (i)	0,00	0,00	0,00	2,01	0,00	5,46	0,00	4,45	0,00	0,29	0,00
C3-Benzoic acid (i)	1,37	0,00	0,00	1,94	0,00	1,96	0,00	0,00	0,00	1,19	0,95
C3-Benzoic acid (i)	3,00	0,00	0,00	12,45	0,00	6,45	0,00	0,00	0,00	0,00	5,83
C3-Benzoic acid (i)	1,72	0,00	0,00	12,10	0,00	2,67	0,00	0,00	0,00	0,00	3,36
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	1,88	0,00	16,27	0,00	3,68	7,23
C3-Benzoic acid (i)	3,64	0,00	0,00	0,00	0,00	1,81	0,00	0,00	0,00	4,45	0,20
C3-Benzoic acid (i)	0,22	0,00	0,00	2,00	0,00	0,00	0,00	0,00	0,00	0,00	0,75
C3-Benzoic acid (i)	1,94	0,00	0,00	2,27	0,00	1,89	0,00	1,55	0,00	0,00	1,85
C3-Benzoic acid (i)	3,16	0,00	0,00	20,98	0,00	1,23	0,00	1,89	0,00	0,00	7,86
C3-Benzoic acid (i)	3,67	0,00	0,00	0,00	0,00	8,85	0,00	5,74	0,00	4,29	3,83
C3-Benzoic acid (i)	1,47	0,00	0,00	2,60	0,00	8,29	0,00	1,25	0,00	0,00	0,78
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	1,67	0,00	0,52	0,00	2,74	0,00
C3-Benzoic acid (i)	8,07	0,00	0,00	0,00	0,00	0,00	0,00	5,56	0,00	0,92	6,40
Fatty acids											
Hexadecanoic acid	22,14	5,68	16,96	27,49	11,42	11,57	11,79	15,28	36,31	6,33	7,38
Octadecanoic acid	13,09	2,73	8,40	17,57	6,57	9,39	12,65	20,92	23,11	4,19	3,93
Sum metabolites	66,81	0,00	0,00	264,24	0,00	119,05	8,66	80,33	0,00	19,45	119,03
Sum Fatty acids	35,23	8,41	25,36	45,06	17,99	20,97	24,44	36,20	59,42	10,51	11,30

(i) = isomers
C2-Benzoic acids: Dimethylbenzoic acid or Ethylbenzoic acid
C3-Benzoic acids: Trimethylbenzoic acid or Methylethylbenzoic acid

Table 22: UST Schäferhof-Süd
Laboratory analytical results for metabolites of BTEX and fatty acids: 02.12.2002

sample	B8	MP1f	MP1t	MP2f	MP2t	MP3f	MP3t	MP4f	MP4t	MP5f	MP5t
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
metabolites											
Benzoic acid	0,00	55,05	14,61	8,91	3,66	3,88	4,51	12,72	3,48	4,5	0,00
Methylbenzoic acid	0,00	0,00	0,00	2,73	0,1	4,74	0,44	21,06	0,28	5,36	11,2
Methylbenzoic acid	0,00	0,00	0,00	9,72	0,07	5,71	0,29	8	0,17	5,96	2,52
Methylbenzoic acid	0,00	0,00	0,00	3,6	0,00	8,47	0,00	0,00	0,00	1,95	0,00
C2-Benzoic acid (i)	5,45	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C2-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	5,1	0,00	0,31	0,00
C2-Benzoic acid (i)	8,39	0,00	0,00	3,26	0,27	1,23	0,00	50,52	0,00	1,31	0,00
C2-Benzoic acid (i)	10,37	0,00	0,00	26,43	0,00	8,27	0,00	27,39	0,08	5,04	32,73
C2-Benzoic acid (i)	0,00	0,00	0,00	11,88	0,05	44,34	0,41	21,39	0,25	4,7	27,99
C2-Benzoic acid (i)	0,00	0,00	0,00	9	0,00	28,92	0,33	25,93	0,00	6	4,39
C2-Benzoic acid (i)	0,00	0,00	0,00	3,62	0,00	8,28	0,00	0,00	0,00	0,00	6,69
C2-Benzoic acid (i)	0,00	0,00	0,00	8,06	0,00	5,02	0,04	12,13	0,48	0,00	0,00
C2-Benzoic acid (i)	3,45	0,00	0,00	8,84	0,00	7,55	0,00	11,38	0,04	0,00	0,00
C2-Benzoic acid (i)	0,00	0,00	0,00	23,77	0,08	25,92	0,34	21,02	0,18	7,25	31,97
C3-Benzoic acid (i)	0,00	0,00	0,00	4,63	0,00	7,66	0,00	0,00	0,00	0,00	0,00
C3-Benzoic acid (i)	3,12	0,00	0,00	3,64	0,00	12,75	0,32	19,93	0,00	2,09	10,94
C3-Benzoic acid (i)	10,37	0,00	0,00	1,88	0,06	7,94	0,24	4,34	0,04	0,65	10,15
C3-Benzoic acid (i)	3,45	0,00	0,00	13,28	0,00	30,72	0,74	15,27	0,00	4,35	26,81
C3-Benzoic acid (i)	0,00	0,00	0,00	5,46	0,00	8,73	0,00	8,89	0,00	1,4	3,68
C3-Benzoic acid (i)	3,62	0,00	0,00	5,07	0,00	11,12	0,00	27,24	0,00	3,05	15,3
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,9	6,88
C3-Benzoic acid (i)	0,00	0,00	0,00	7,57	0,00	26,96	0,00	19,61	0,00	4,54	8,77
Fatty acids											
Tetradecanoic acid	1,96	36,2	11,8	6,64	4,56	12,05	5,87	0,00	5,94	4,98	9,68
Hexadecanoic acid	8,67	129,45	30,37	18,68	13,51	21,61	14,43	20,62	13,41	14,12	8,44
Octadecanoic acid	4,34	71,21	15,49	7,12	7,89	11,64	6,88	9,37	7,43	6,28	5,14
Sum metabolites	48,22	55,05	14,61	161,35	4,31	258,22	7,66	311,91	5,01	59,34	200,02
Sum Fatty acids	14,97	236,85	57,66	32,44	25,96	45,3	27,18	29,99	26,78	25,37	23,26

(i) = isomers
C2-Benzoic acids: Dimethylbenzoic acid or Ethylbenzoic acid
C3-Benzoic acids: Trimethylbenzoic acid or Methylethylbenzoic acid

Table 23: UST Schäferhof-Süd
Laboratory analytical results for metabolites of BTEX and fatty acids: 03.06.2003

sample	B8f	B8t	MP1f	MP1t	MP2f	MP2t	MP3f	MP3t	MP4f	MP4t	MP5f	MP5t
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
metabolites												
Benzoic acid	2,90	2,78	2,14	9,58	6,84	2,88	6,72	4,02	7,60	2,18	2,82	3,00
Methylbenzoic acid	15,02	14,40	0,00	0,00	3	0,18	3,28	0,00	17,06	0,08	7,06	12,62
Methylbenzoic acid	6,38	4,34	0,20	0,72	10,92	0,42	4,42	0,54	14,86	0,24	2,48	8,84
Methylbenzoic acid	2,78	1,68	0,00	0,00	17,62	0,18	2,90	0,18	6,90	0,10	1,46	4,94
C2-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	6,32	0,00	0,00	0,00	0,00	1,94
C2-Benzoic acid (i)	39,50	24,78	0,00	0,00	103,28	0,03	23,54	0,00	25,02	0,12	7,30	18,28
C2-Benzoic acid (i)	15,92	12,16	0,00	0,00	32,54	0,00	6,92	0,00	8,84	0,00	3,92	9,02
C2-Benzoic acid (i)	37,70	22,10	0,00	0,00	72,8	0,30	14,66	0,00	25,06	0,00	7,80	24,82
C2-Benzoic acid (i)	8,60	6,24	0,00	0,00	9,62	0,00	8,22	0,00	7,18	0,00	0,96	6,28
C2-Benzoic acid (i)	5,90	6,20	0,00	0,00	0,00	0,00	0,00	0,00	16,30	0,00	1,42	7,90
C2-Benzoic acid (i)	8,58	0,00	0,00	0,00	0,00	0,00	4,30	0,00	10,16	0,00	0,00	6,66
C2-Benzoic acid (i)	12,68	3,42	0,00	0,00	54,42	0,00	7,94	0,00	0,00	0,00	1,54	9,12
C2-Benzoic acid (i)	24,58	26,48	0,00	0,00	66,22	0,00	40,28	0,14	44,76	0,00	4,76	12,48
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	11,14	0,00	0,00	0,00	10,30	0,00	0,46	1,26
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	3,46	12,96
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	53,70	0,00	9,74	0,00	21,28	0,03	2,80	2,92
C3-Benzoic acid (i)	2,68	6,56	0,00	0,00	18,96	0,00	6,52	0,00	6,32	0,00	0,00	8,02
C3-Benzoic acid (i)	34,66	0,00	0,00	0,00	106,66	0,22	14,06	0,00	14,62	0,00	4,44	4,46
C3-Benzoic acid (i)	15,00	26,32	0,00	0,00	50,98	0,00	3,70	0,00	0,00	0,00	0,00	5,28
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	15,14	0,00	0,00	0,00	0,00	0,00	0,00	3,06
C3-Benzoic acid (i)	0,00	19,00	0,00	0,00	11,62	0,00	0,00	0,00	0,00	0,00	0,00	22,60
C3-Benzoic acid (i)	0,00	12,48	0,00	0,00	54,58	0,28	5,94	0,00	0,00	0,00	2,92	4,74
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	3,8	0,00	4	0,00	0,00	10,90
Fatty acids												
Tetradecanoic acid	0,00	0,00	3,90	19,32	0,00	5,68	9,96	6,48	0,00	2,22	5,18	4,24
Hexadecanoic acid	9,16	6,54	8,38	60,32	14,98	18,26	52,74	26,64	19,2	6,12	11,48	12,04
Octadecanoic acid	6,38	0,00	22,72	42,38	8,94	3,4	28,22	19,18	16,14	22,34	6,70	7,68
Sum metabolites	232,88	188,94	2,34	10,30	700,04	4,49	173,26	4,88	240,26	2,75	55,6	202,10
Sum Fatty acids	15,54	6,54	35,00	122,02	23,92	27,34	90,92	52,3	35,34	30,68	23,36	23,96

(i) = isomers
C2-Benzoic acids: Dimethylbenzoic acid or Ethylbenzoic acid
C3-Benzoic acids: Trimethylbenzoic acid or Methylethylbenzoic acid

Table 24: UST Schäferhof-Süd
Laboratory analytical results for metabolites of BTEX and fatty acids: 04.12.2003

sample	MP1f	MP1t	MP2f	MP2t	MP3f	MP3t	MP4f	MP4t	MP5f	MP5t
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
metabolites										
Benzoic acid	0,67	0,88	3,13	2,63	2,59	3,16	0,00	0,19	0,00	0,64
Methylbenzoic acid	0,00	0,00	0,00	0,00	1,07	0,00	18,08	0,00	9,86	16,52
Methylbenzoic acid	0,00	0,00	2,92	0,03	1,18	0,00	8,56	0,00	3,77	7,29
Methylbenzoic acid	0,00	0,00	0,81	0,00	0,70	0,00	4,65	0,02	0,77	7,82
C2-Benzoic acid (i)	0,00	0,00	2,01	0,00	8,08	0,00	34,73	0,00	5,01	30,53
C2-Benzoic acid (i)	0,00	0,00	1,42	0,00	4,39	0,00	8,55	0,13	4,22	11,68
C2-Benzoic acid (i)	0,00	0,00	4,27	0,00	0,00	0,00	0,00	0,00	0,00	48,41
C2-Benzoic acid (i)	0,00	0,00	7,37	0,00	7,75	0,00	16,87	0,07	8,62	0,00
C2-Benzoic acid (i)	0,00	0,00	0,00	0,00	2,12	0,00	7,86	0,00	2,52	9,87
C2-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	10,69	0,04	8,19	11,04
C2-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	11,51	0,00	0,00	5,01
C2-Benzoic acid (i)	0,00	0,00	4,61	0,00	0,00	0,00	0,00	0,00	4,03	12,00
C2-Benzoic acid (i)	0,00	0,00	21,73	0,00	34,44	0,14	11,75	2,28	3,75	20,05
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,10	0,00	10,53
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	18,25	0,00	1,19	3,67
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	2,96	0,00	7,29	0,09	3,97	16,60
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	1,52	0,00	3,02	0,00	5,28	5,64
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	6,83	0,00	8,69	0,00	7,83	25,42
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	1,64	0,00	4,77	0,00	4,87	0,00
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,99	0,00	0,00	0,00	0,00	5,67
C3-Benzoic acid (i)	0,00	0,00	4,09	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Fatty acids										
Tetradecanoic acid	2,17	2,35	13,24	2,75	6,59	3,86	0,00	2,16	6,60	0,00
Hexadecanoic acid	9,75	9,25	30,73	12,23	15,08	18,12	26,44	8,96	18,71	8,07
Octadecanoic acid	7,77	7,84	24,72	8,48	8,35	22,68	20,41	8,95	12,44	5,46
Sum metabolites	0,67	0,88	52,37	2,66	76,24	3,30	175,26	2,92	73,89	248,40
Sum Fatty acids	19,69	19,44	68,69	23,47	30,03	44,66	46,85	20,08	37,76	13,54

(i) = isomers
C2-Benzoic acids: Dimethylbenzoic acid or Ethylbenzoic acid
C3-Benzoic acids: Trimethylbenzoic acid or Methylethylbenzoic acid

Table 25: UST Schäferhof-Süd
Laboratory analytical results for metabolites of BTEX and fatty acids: 08.06.2004

sample	MP1f	MP1t	MP2f	MP2t	MP3f	MP3t	MP4f	MP4t	MP5f	MP5t
	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l	µg/l
metabolites										
Benzoic acid	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Methylbenzoic acid	0,00	0,00	0,00	0,00	0,00	0,00	27,45	0,00	0,00	0,00
Methylbenzoic acid	0,00	0,00	0,00	0,00	0,00	0,00	18,09	0,00	0,00	0,00
Methylbenzoic acid	0,00	0,00	0,00	0,00	0,00	0,00	5,81	0,00	0,00	0,00
C2-Benzoic acid (i)	0,00	0,00	12,90	0,00	0,00	0,00	10,20	0,00	0,00	19,70
C2-Benzoic acid (i)	0,00	0,00	3,14	0,00	0,00	0,00	3,57	0,00	2,42	4,51
C2-Benzoic acid (i)	0,00	0,00	3,42	0,00	5,30	0,00	29,93	0,00	0,00	2,07
C2-Benzoic acid (i)	0,00	0,00	3,38	0,00	1,35	0,00	0,00	0,00	0,00	10,44
C2-Benzoic acid (i)	0,00	0,00	1,99	0,00	5,41	0,00	10,37	0,00	0,39	5,79
C2-Benzoic acid (i)	0,00	0,00	0,56	0,00	3,90	0,00	17,85	0,00	1,59	14,88
C2-Benzoic acid (i)	0,00	0,00	1,06	0,00	8,97	0,00	0,00	0,00	1,11	1,21
C2-Benzoic acid (i)	0,00	0,00	0,89	0,00	5,35	0,00	12,81	0,00	0,95	1,62
C2-Benzoic acid (i)	0,00	0,00	3,62	0,00	6,94	0,00	35,92	0,00	2,28	6,77
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	13,87	0,00	0,00	8,01
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	4,87	0,00	0,00	0,00	0,00	1,17
C3-Benzoic acid (i)	0,00	0,00	1,65	0,00	0,00	0,00	13,09	0,00	0,00	1,96
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	7,76	0,00	5,09	0,00	0,00	20,98
C3-Benzoic acid (i)	0,00	0,00	1,88	0,00	13,00	0,00	0,00	0,00	0,00	0,00
C3-Benzoic acid (i)	0,00	0,00	7,22	0,00	0,00	0,00	13,56	0,00	0,00	0,00
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,64	8,02
C3-Benzoic acid (i)	0,00	0,00	3,69	0,00	0,00	0,00	0,00	0,00	1,07	8,09
C3-Benzoic acid (i)	0,00	0,00	9,00	0,00	0,00	0,00	24,98	0,00	5,44	21,01
C3-Benzoic acid (i)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Fatty acids										
Tetradecanoic acid	0,00	0,00	4,04	0,00	0,00	1	0,00	0,00	0,00	0,00
Hexadecanoic acid	0,00	0,00	13,64	0,33	21	5	27	0,82	17,34	0,00
Octadecanoic acid	0,00	0,00	9,10	0,50	12	6	35	0,00	10,71	0,00
Sum metabolites	0,00	0,00	54,40	0,00	62,85	0,00	242,59	0,00	15,89	136,23
Sum Fatty acids	0,00	0,00	26,78	0,83	33,21	11,92	61,73	0,82	28,05	0,00

(i) = isomers
C2-Benzoic acids: Dimethylbenzoic acid or Ethylbenzoic acid
C3-Benzoic acids: Trimethylbenzoic acid or Methylethylbenzoic acid

Table 26: UST Schäferhof-Süd
Laboratory analytical results for sediment sampling at the drilling points B3 and B5 in Sep. 2004

depth	B3/3	B3/4	B5/3	B5/4
[m]	BTEX mg/kg	BTEX mg/kg	BTEX mg/kg	BTEX mg/kg
0-1	0	5	1.3	0
1-2	0	0	2.2	0
2-3	0	0	11	2
3-4	0	12	3.8	8.6
4-5	17	14	11	5.3
5-6	120	87	66	46
6-7	4	6.7	2.2	4
7-8	1.3	4.1	0	6.3

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