Heterocyclic compounds in tar oil contaminated groundwater

- occurrence, fate, and indications for natural attenuation -

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ABBREVIATIONS

AQDS	anthraquinone-2,6-disulphonic acid
bls	below top ground surface
BTEX	benzene, toluene, ethylbenzene, xylene
c_s	concentration (mg/kg) sorbed to montmorillonite or aquifer material
c_{aq}	concentration (mg/L) in the aqueous solution
DAD	diode array detector
DNAPL	dense non-aqueous phase liquids
FID	flame ionisation detector
FLD	fluorescence detector
GC	gas chromatography
HPLC	high performance liquid chromatography
K_d	distribution coefficient
K_f	hydraulic conductivity, permeability of the aquifer
K_F	Freundlich distribution coefficient
$K_{tar\ oil/water}$	tar oil-water partition coefficient
KORA	research funding priority “Retention and Degradation Processes to Reduce Contaminations in Groundwater and Soil”
K_{OW}	octanol-water partition coefficient
LOEC	lowest observed effect concentration
LOD	limit of detection
log P_{OW}	octanol/water partition coefficient
LOQ	limit of quantification
MHE	multiple headspace extraction
MS	mass spectrometry
m/z	mass/charge ratio
NA	natural attenuation
NAPL	non-aqueous phase liquids
NOEC	no observed effect concentration
n.d.	not determined
PAHs	polycyclic aromatic hydrocarbons
pK_a	acid dissociation constant
PNEC	predicted no effect concentration
QSAR	quantitative structure-activity relationship
r_t	retention time (min)
SCAN-mode	total ion monitoring
SIM-mode	selected ion monitoring

ABSTRACT

Former coke manufacture, gasworks as well as timber impregnation sites are contaminated by tar oils. The sources of such contamination include the leakage of storage tanks, poor past practices such as on-site disposal, and ineffective decommissioning of the plants. Besides PAHs, which are the main ingredients of tar oils with about 85 %, and 1-10 % phenolic compounds, NSO-heterocyclic compounds are present at about 5-13 %. However, NSO-heterocycles which are strongly smelling and toxic compounds are seldom determined at these sites, although they are characterized by high mobility because of their high water solubility and low sorptive behaviour.

The contaminated sites are often large and a complete clean-up is difficult in terms of money and technology. Due to the associated environmental and potential human health risks, the development of efficient and cost-effective remediation approaches is urgently needed, to manage the risks posed by subsurface contamination in these sites. Besides other strategies the concept of natural attenuation relies on the reduction of contamination in the ground.

The aim of this thesis was to assess the relevance of NSO-heterocycles in the groundwater of three tar oil contaminated sites and furthermore to search for indications for the presence of Natural Attenuation processes including the group of NSO-heterocycles.

Therefore, as a first step, analytical methods for groundwater analyses were developed. In addition to an existing HPLC-DAD method for the detection of a huge number of NSO-heterocycles, a LC-MS-MS method for a selective and sensitive analysis of N-heterocyclic compounds as well as a Headspace-GC-MS method for the analysis of volatile analogs was performed.

The site-directed survey allowed the long distance comparison of the concentration of various NSO-heterocyclic compounds. Differences in distribution and decrease over the field sites using mass loads were discussed in context to compounds physicochemical characteristics to work out indications for microbial natural attenuation processes of NSO-heterocycles. N-heterocycles like quinoline compounds were found in high concentrations near the source of contaminated at all of the three sites. In comparison to S- and O-heterocycles a higher decrease of quinoline compounds with the flow line of groundwater was observed. Methylsubstituted S- and O-heterocycles were found of special relevance downstream the source. A comparison of these heterocycles with EPA-PAHs, especially with acenaphthene, which is known as a highly persistent PAH, shows the necessity to consider these compounds.

To cover indications of biodegradation from the field investigation, different degradation studies were performed: Microcosms were carried out by using groundwater with the authentic contamination from the Düsseldorf-Flingern and Wülknitz site to study microbial degradation of NSO-heterocycles within up to two years. The influence of variation of redox conditions as well as addition of reducing agents was studied. In addition, the microbial activity in an *on-site* column filled with aquifer material and contaminated groundwater from the Wülknitz field site was analysed. This approach allowed the detection of a decrease in concentration within a reasonable time period of only several weeks. The high-resolution well at Düsseldorf-Flingern provided an insight into the micro distance comparison of the concentration of various contaminants as well as electron acceptors such as sulfate across the fringe and centre zones and therefore a comparison of different contaminants plumes. Indications for the presence of microbial degradation in the plumes fringe could be worked out.

Altogether, data of contaminants distribution from groundwater analyses, of decrease in concentration in degradation experiments and of the plumes width in the high-resolution well were critically interpreted. The decrease in concentration along the plumes together with the

results from the more artificial degradation experiments and the high-resolution well led to the general conclusion that biodegradative processes towards heterocycles and homocycles are present in the aquifers. Especially O- and S-heterocyclic compounds as well as their methylated analogs were found of high relevance downstream the source of contamination, and showed persistence in the degradation experiments.

In the second part of the thesis the possibility to use the occurrence of anaerobic degradation products for the demonstration of biodegradation at the field sites was investigated. The aim was to explore whether hydroxylated quinoline and isoquinoline or methylquinoline found in the groundwater of the three field sites in high concentrations can be used as indicator for natural attenuation. Therefore, the degradative potential of aquifer material towards compounds in single, quinoline, isoquinoline as well as methylsubstituted quinolines and the formation of metabolites was studied. Hydroxylated and hydrogenated metabolites were formed in high amounts and accumulated, especially under sulfate-reducing conditions, the prevailing conditions in the field. However, the analyses of seven tar products showed that these hydroxylated compounds, 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone, 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone are also primary constituents. Therefore, their detection in groundwater was found to be a non-sufficient indicator for the occurrence of biological natural attenuation processes. Instead, the ratio of hydroxylated to parent compound ($R_{metabolite}$) was tested as a useful indicator.

All parameters which may influence this ratio had to be studied: Dilution in a plume occurred the same way for both type of compounds, and volatilization of these was negligible, because of low Henry's law constants. Both parameters did not shift the ratio found in the tar products. The main parameters besides biological degradative processes, which might influence $R_{metabolite}$, were seen in differences in sorption and dissolution from tar oils of the parent and hydroxylated quinoline compound. Sorption was found to influence both partners of $R_{metabolite}$ in a minor extend. The main parameter influencing the ratio was found in differences in partitioning of parent and hydroxylated quinoline compound between the tar oil and water phase. With respect to the observed partition coefficient between tar oil and water of 3.5 for quinoline and isoquinoline and 0.3 for 2(1H)-quinolinone and 1(2H)-isoquinolinone, the ratio in groundwater would be approximately 10 times higher than the ratio in tar oil. When paying attention to these parameters and including a sufficient threshold factor, in a broad number of groundwater samples of all three investigated sites the highest ratio found in tar oils was clearly exceeded. This indicates that biological processes take place in the aquifer of the three sites and $R_{metabolite}$ is an applicable indicator for the presence of natural attenuation. The $R_{metabolite}$ concept was found to be feasible for the couples 2(1H)-quinolinone/quinoline, 3,4-dihydro-2(1H)-quinolinone/quinoline, 1(2H)-isoquinolinone/isoquinoline, and also for methylsubstituted quinolines and their hydroxylated metabolites.

The influence of microbial degradation of N-heterocyclic compounds on ecotoxic, mutagenic, and genotoxic potential of these compounds was studied. The results showed that several hydroxylated quinoline metabolites were of toxicological relevance in addition to the parent compounds.

ZUSAMMENFASSUNG

Das Grundwasser ehemaliger Kokereien, Gaswerke oder Holzimprägnierwerke ist häufig durch unsachgemäßen Umgang in der Vergangenheit, Leckagen, Zerstörungen während des Zweiten Weltkrieges wie auch unsachgemäßen Abbruch dieser Standorte, mit Teerölen belastet. Neben polyzyklischen aromatischen Kohlenwasserstoffen, die mit 85 % den Hauptanteil in Teerölen ausmachen, kommen neben phenolischen Verbindungen (1-10 %) auch sog. NSO-Heterocyclen mit 5-13 % vor. Obwohl es sich bei NSO-Heterocyclen aufgrund ihrer hohen Wasserlöslichkeit und geringen Sorptionstendenz um sehr mobile Teerölverbindungen handelt, die sehr geruchsaktiv sind und – wenn bekannt – ein hohes toxisches Potential besitzen, werden sie bislang nicht in Routineuntersuchungen des Grundwassers von Teerölaltlastenstandorten einbezogen.

Teerölaltlastenstandorte umfassen meist großflächige Gebiete, so dass eine komplette Dekontamination durch Sanierung selten praktikabel und finanzierbar ist. Aufgrund der mit der Teerölbelastung verbundenen potentiellen Gefahr für Mensch und Umwelt ist eine effiziente und kosteneffektive Sanierungsstrategie notwendig. Das Natural Attenuation-Konzept setzt auf die im Untergrund ablaufenden Prozesse der Schadstoffminderung.

Das Ziel dieser Arbeit war es, die Relevanz der NSO-Heterocyclen im Grundwasser von drei Teerölaltlastenstandorten herauszuarbeiten und Hinweise auf Natural Attenuation-Prozesse in Bezug auf die Gruppe der NSO-Heterocyclen zu suchen.

Dazu wurden zunächst entsprechende Methoden entwickelt, um Grundwasserproben analysieren zu können. Zusätzlich zu einer bisher bestehenden HPLC-DAD-Methode zur Untersuchung einer großen Zahl an verschiedenen NSO-Heterocyclen, wurde eine HPLC-MS-MS-Methode für die Analyse von N-Heterocyclen sowie eine Headspace-Methode für die Untersuchung von leichtflüchtigen NSO-Heterocyclen im Grundwasser entwickelt.

Die Felduntersuchungen erlaubten es, die Verteilung und den Verbleib der NSO-Heterocyclen über eine lange Feldstrecke zu beobachten. Unterschiede in der Verteilung und Abnahme über die Feldstrecke unter Nutzung des Aufstellens von Massenbilanzen werden im Zusammenhang mit physikochemischen Eigenschaften der verschiedenen Verbindungen diskutiert, um Hinweise auf mikrobielle Natural Attenuation-Prozesse anhand der Gruppe der NSO-Heterocyclen herauszustellen. N-Heterocyclen wie verschiedene Chinoline zeichneten sich dabei an allen drei Standorten durch sehr hohe Konzentrationen quellennah aus. Eine deutlich höhere Abnahme mit dem Grundwasserstrom war für Chinolinverbindungen im Vergleich zu S- und O-Verbindungen erkennbar. Besonders zeigte sich die Bedeutung von methylierten S- und O-Heterocyclen im Grundwasserabstrom der Standorte. Ein Vergleich mit den EPA-PAKs und konkret der als sehr persistent bekannten PAK-Verbindung Acenaphthen zeigte, dass diesen Verbindungen genauso viel Beachtung geschenkt werden sollte.

Um generelle Hinweise auf mögliche anaerobe mikrobielle Abbauvorgänge und Unterschiede in der Abbaubarkeit der Heterocyclen zu erlangen und herauszuarbeiten, wurden unterschiedliche Abbauversuche durchgeführt: Mikrokosmen mit Grundwasser des Standortes Düsseldorf-Flingern und Wülknitz mit der authentischen Belastung wurden bis zu zwei Jahre auf die Abbaubarkeit der NSO-Heterocyclen untersucht, wobei der Einfluss verschiedener Redoxbedingungen und Reduktionsmittel ermittelt wurde. Weiter wurde der anaerobe Abbau der Heterocyclen im Vergleich zu ausgewählten PAK in zwei *on-site* Säulen, gefüllt mit Aquifermaterial und Grundwasser des Standortes Wülknitz, untersucht, wobei hier innerhalb eines Zeitraumes von nur wenigen Wochen Unterschiede in der mikrobiellen Abbaubarkeit der Verbindungen aufgezeigt werden konnten. Zusätzlich konnten durch Grundwasserbeprobungen im kleinsten Maßstab, mittels eines hochauflösenden Brunnens am Standort Düsseldorf-Flingern, klare Schadstofffahnen der verschiedenen Verbindungen

erkannt werden, deren Vergleich untereinander und mit Elektronenakzeptoren, insbesondere Sulfat, Hinweise auf mikrobielle Abbauvorgänge ermöglichte.

Das Ziel des zweiten Teils dieser Arbeit war es zu untersuchen, ob Abbauprodukte des anaeroben mikrobiellen Abbaus wie hydroxylierte Chinoline und Isochinoline, die in hohen Konzentrationen im Grundwasser der drei Standorte vorkommen, als Indikator für biologische Natural Attenuation-Prozesse genutzt werden können. Dazu wurde zunächst unter anaeroben Redoxbedingungen die Abbaubarkeit von Chinolin, Isochinolin und Methylchinolinen und die Entstehung von Metaboliten untersucht. Hydroxylierte Metabolite sowie deren reduzierte Analoga akkumulierten meist in hohen Konzentrationen, besonders unter sulfatreduzierenden, den im Feld vorherrschenden Bedingungen. Untersuchungen von verschiedenen Teerölen zeigten jedoch, dass die oben angesprochenen Metabolite auch selbst Teerölbestandteile darstellen. Daher ist es nicht möglich, das alleinige Vorkommen dieser Verbindungen im Grundwasser als Hinweis auf biologische Natural Attenuation-Prozesse zu bewerten. Als Ausweg wird hier die Nutzung des Verhältnisses zwischen der jeweiligen hydroxylierten Verbindung und der Ausgangsverbindung ($R_{metabolite}$) vorgeschlagen, da dieses, wie in den Abbauversuchen nachgewiesen, entscheidend von mikrobiellem Abbau bestimmt wird.

Die verschiedenen Parameter, die dieses Verhältnis ($R_{metabolite}$) neben mikrobiellen Abbauprozessen beeinflussen, wurden untersucht: Verdünnung findet sowohl für die hydroxylierte wie auch die Ausgangsverbindung in gleichem Maße statt. Auch eine Verschiebung des Verhältnisses durch unterschiedliche Verflüchtigung kann für beide Verbindungsgruppen ausgeschlossen werden, da die Henry-Koeffizienten ausgesprochen niedrig sind. Sowohl für die hydroxylierte Verbindung wie auch die Ausgangsverbindung wurde nur eine sehr geringe Sorption am Aquifermaterial festgestellt, die eine Verschiebung des Verhältnisses im Grundwasser nicht beeinflusst. Ein wesentlicher Faktor, der zu einer Verschiebung des Verhältnisses auf die Seite der hydroxylierten Verbindung in der Schadstoffquelle führt, ist die verschiedene Verteilung zwischen der Teerölphase und der Wasserphase. Für Chinolin und Isochinolin wurde ein Verteilungskoeffizient zwischen der Teeröl- und Wasserphase von 3,5, für 2(1H)-Chinolinon und 1(2H)-Isochinolinon von nur 0,3 bestimmt. Damit ist im Grundwasser ein um den Faktor 10 höheres Verhältnis ($R_{metabolite}$) zu erwarten als das Verhältnis im ursprünglichen Teeröl. Wird dieser Faktor in die Untersuchungen der Verhältnisse im Grundwasser der Standorte einbezogen, zeigt das Vorhandensein vieler Grundwasserproben in denen dieses Grenzverhältnis deutlich überschreiten ist an, dass mikrobielle Abbauprozesse bezüglich der Chinolinverbindungen am Standort ablaufen. Daher wurde der Verhältnis-Ansatz als eine gute Möglichkeit befunden, biologische Natural Attenuation Prozesse der Chinolinverbindungen im Feld aufzuzeigen. Das $R_{metabolite}$-Konzept wurde als sinnvoll für die Verbindungspaare 2(1H)-Chinolinon/ Chinolin, 3,4-Dihydro-2(1H)-chinolinon/Chinolin, 1(2H)-Isochinolinon/Isochinolin, sowie auch für methylsubstituierte Chinoline und ihre hydroxylierten Metaboliten gefunden.

Untersuchungen des mikrobiellen Umsatzes von Chinolinverbindungen zu ihren hydroxylierten Analoga zeigten im Hinblick auf Ökotoxizität, Genotoxizität und Mutagenität neben der Relevanz der Ausgangsverbindungen auch die Bedeutung der hydroxylierten Verbindungen.

1. INTRODUCTION

1.1 Tar oil contaminated sites

A high number of former industrial sites is known worldwide to be contaminated with tar oils or creosotes. Diverse contaminations at varying extent resulted from the production and/or the usage of these complex mixtures arising from:

- coke manufactures,
- gasworks, and
- wood-preserving facilities.

Coke or town-gas manufactures produce coal tars in the high temperature carbonisation of coal. Further fractional distillation of these products in the temperature range of 200-400 °C leads to brownish-black, oily liquids, called tar oil or creosote (Figure 1). The production of these by-products was closely linked to the steel industry, because of the need of coke in the high-temperature process. The other important source of coal tar in former times was the gas manufacture from coal. Gas manufacture is rapidly declined since the 1960s to 1970s due to increasing exploitation of natural gas (IARC, 1985; Heikkilä, 2001). However, until now these former sites are highly contaminated with tar oils. In Germany the number of coke manufacturing and gasworks sites, where the ground is highly contaminated, is estimated of about 500 (Schmitt et al., 1998).

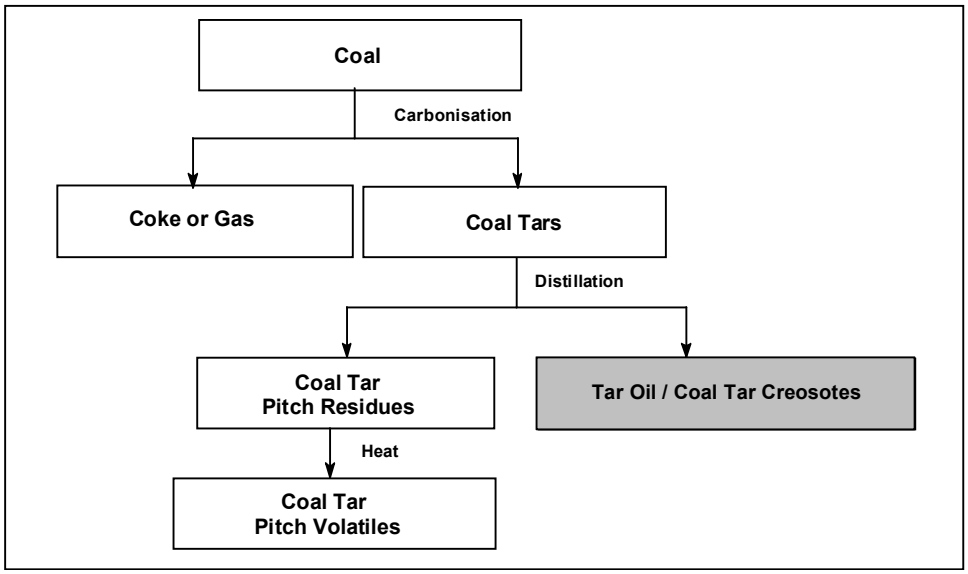


Figure 1: Formation of creosote from coal tar distillation (according to WHO, 2004).

Until now, tar oils are commonly used as wood preservative worldwide, and comprise nearly 15 % of the total volume of wood treatment preservatives used in the United States (Crawford et al., 2000). Tar oil is an effective fungicide, insecticide, and sporicide used as a wood preservative for above and below ground wood protection treatments as well as for treating wood in the marine environment. It has been estimated that there are 700 sites throughout the United States where wood preservation is, or has been, conducted. They reportedly consume approximately 4.5×10^7 kg of tar oil annually (Mueller et al., 1989). In Germany approx. 3×10^7 kg of tar oils were produced per year in 1998 (Böhm et al., 2002). However, in Germany the usage of tar oils is only allowed for the impregnation of timber, railway sleepers and telegraph poles or power poles (ChemVerbotsV, 2003).

Highly contaminated surface soils, waters in treatment lagoons or evaporation areas, and groundwaters contaminated with leachate from the above sources resulted from the misuse,

leakage of storage tanks, drippings from treated lumber, spills, and leachate from unlined holding ponds, improper disposal, and accidental spillage. Therefore, serious potential health risks from the coke manufacturing, the gasification or wood impregnation sites were expected.

1.2 Tar oils

Tar oil, a bulk distillate of coal tar, is a complex mixture of over 200 major individual compounds with varying molecular weights, polarities, and functionalities along with dispersed solids and products of polymerization. The exact composition depends on the parent coal, the production process of the coal tar as well as the temperature used for production and fractionation. The mixture of tar oils contains only limited compounds (less than 20 %), which are present in amounts greater than 1 % (Lorenz and Gjovik, 1972; Nylund et al., 1992). The chemical structures of some of these constituents are given in Figure 2.

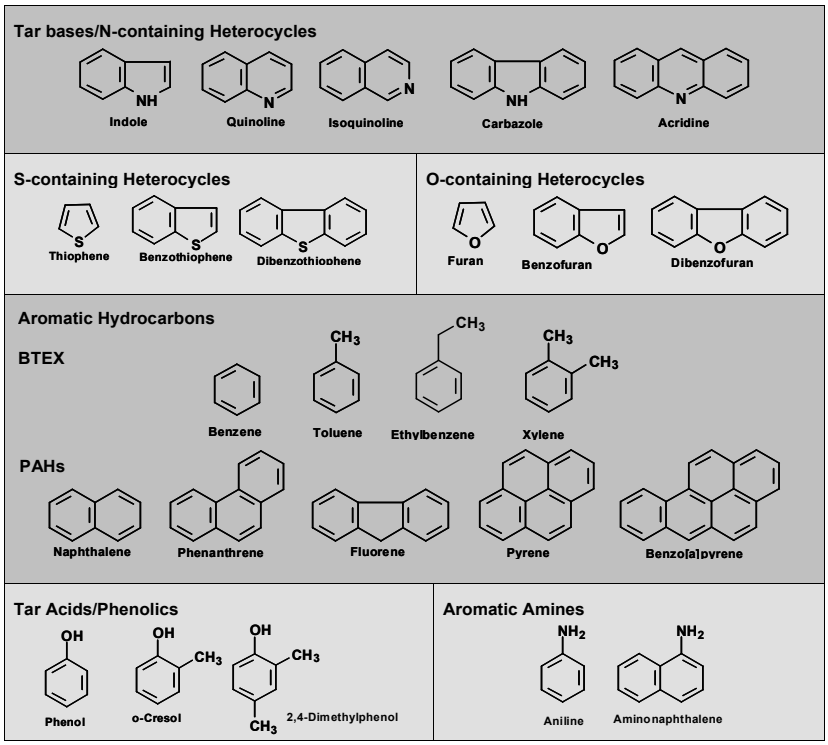


Figure 2: Chemical structures of some tar oil constituents (according to WHO, 2004).

The six major classes of compounds present in tar oils are the following (Willeitner & Dieter, 1984; U.S. EPA, 1987):

- *aromatic hydrocarbons*: PAHs and alkylated PAHs, which can constitute up to 90 % of tar oil, and <1 % BTEX (Meyer et al., 1999)
- *tar acids* / *phenolics*: phenols, cresols, xylenols, and naphthols. Tar acids are present up to 1–3 %; phenolics 2 –17 % (Bedient et al., 1984)
- *tar bases / nitrogen-containing heterocycles*: pyridines, quinolines, benzoquinolines, acridines, indolines, and carbazoles. Tar bases form up to 1–3 %; nitrogen-containing heterocycles, 4.4– 8.2 % (Heikkilä, 2001)
- *aromatic amines:* aniline, aminonaphthalenes, diphenylamines, aminofluorenes, and aminophenanthrenes (Wright et al., 1985), as well as cyano-PAHs, benzacridine, and its methylsubstituted congeners (Motohashi et al., 1991)
- *sulfur-containing heterocycles:* benzothiophenes and their derivatives (1–3 %), and

- *oxygen-containing heterocycles*: benzofurans, dibenzofurans (5–7.5 %)

The ratio of N:S:O-heterocycles is approximately 7:2:1 (Collin and Zander, 1985). General amounts of selected compounds in coal tars are summarized in Table 1.

1.3 Transport and distribution of tar oil in an aquifer

The transport, transformation, and accumulation of the components of creosote in an aquifer are strongly influenced by the components' physical and chemical properties, their interactions in the mixture and interactions with the aquifer/soil matrix. As a result of the chemical complexity the behaviour of individual components in the environment may not reflect the behaviour of a compound if it is a component of tar oil.

"Pure" tar oil is denser than water. In the aquifer tar oil can occur as lighter- and heavier-than-water fractions, and consequently can be found above and below the water table, or even as a free liquid pool. Because of the higher density (1.00–1.17 g/m^3 at 25 °C; WHO, 2004) the heavier fraction of tar oils tends to travel downwards the aquifer until it encounters an impervious layer and forms so called DNAPLs (dense non-aqueous phase liquids) (Zheng et al., 2001; Barranco and Dawson, 1999; Pereira et al., 1983). The heavier fraction, will flow along the impervious interface through the more porous soil in the downslope direction. The light fraction includes most of the nitrogen-, oxygen-, and sulfur-substituted heterocyclic components, naphthalenes, acenaphthene, fluorene, phenols. With increasing dwell time of a tar oil in an aquifer, the viscosity of the tar oil contamination increases because of the depletion of highly water soluble compounds. Less water soluble compounds remain in the mixture (Schüth, 1994; Arvin et al., 1988). The light fraction components, because of being more water soluble than the other tar oil components, will be dissolved and transported in the groundwater. The light fraction has also been found to move with fluctuating water levels and, as a result, can contaminate the whole soil layer. Therefore, compounds of low polarity are of long-time importance, while highly water soluble compounds are of highly relevance for the assessment of groundwater quality downstream the plume (WHO, 2004).

PAHs form the highest amount in tar oils. In contrast, NSO-heterocyclic compounds (Table 22, appendix) due to the high water solubility and weak sorption tendency can reach up to 35-40 % of the water-soluble fraction of tar oils and therefore are potential groundwater contaminants (Licht et al., 1996), while PAHs form only up to 20 % of the water-soluble fraction (Arvin et al., 1988; Meyer and Steinhart, 2000).

In Table 1 compounds investigated in this study - several hetero- as well as homocyclic compounds - are summarized and their amount in tar products as well as range of concentration found in groundwater of tar oil contaminated sites are shown.

Table 1: Occurrence of selected hetero- and homocyclic compounds in coal tars and in tar oil contaminated groundwater.

compound	occurrence in coal tar creosote [%]	maximum concentration found in tar oil contaminated groundwater [µg/L]
N-Heterocycles		
quinoline	0.3[1]–2.0[2]	11200[b]
isoquinoline	0.2[1]–0.7[2]	5400[c]
carbazole	0.22[3]–3.9[2]	30420[a]
acridine	0.2[3]–2[2]	4110[a]
S-Heterocycles		
benzothiophene	0.3[1]–0.5[3]	2480[c]
dibenzothiophene	0.3[1]–1.0[4]	55980[a]
O-Heterocycles		
benzofuran	<0.1[3]–0.5[1]	490[f]
dibenzofuran	1.0[1]–7.5[5]	84420[a]
Homocycles/PAHs		
naphthalene	1.3[5]–18[6]	26500[f]
1-methylnaphthalene	0.5[1]–7[6]	29110[c]
2-methlnaphthalene	0.5[1]–12[6]	2860[a]
dimethylnaphthalene	1.6[4]–5.6[7]	8810 (2,3-isomer)[a]
indene	0.6[3]–0.87[3]	1310[e]
fluorene	2.0[1]–10[5]	141330[a]
acenaphthene	2.0[1]–14.7[5]	139760[a]

[1]Collin and Zander, 1985; [2]Wright et al., 1985; [3]Nylund et al., 1992; [4]ITC, 1990; [5]Lorenz and Gjovik, 1972; [6]Andersson and Lovley, 1999; [7]Nestler, 1974; [a]Mueller et al., 1993; [b]Ondrus and Steinheimer, 1990; [c]Middaugh et al., 1994; [d]Johansen et al., 1997b; [e]Goerlitz et al., 1985.

1.4 Toxicity of heterocyclic compounds

While there exists a lot of information about the toxicity of PAHs, only few studies deal with the toxicity of NSO-heterocyclic compounds, hence an urgent need exists to include heterocyclic compounds as well as their transformation products into toxicological studies (Bundy et al., 2001; Droge et al., 2006).

Known or possible carcinogens in tar products are benzene as well as some of the EPA-PAHs. As reference component usually benzo[a]pyrene is used, which shows extremely high carcinogenic potential. However, studies investigating the carcinogenic potential of tar products found a potential about 5 times higher than expected to occur from the amount of benzo[a]pyrene present in the tar oil (CSTEE, 1999). Therefore, the presence of some other compounds of high toxicological relevance was indicated.

Wilson et al. (1981) as well as Later et al. (1982) found that especially the N-heterocycles in tar oils are of highest potential to act as mutagens. The proof on mutagenicity in the Ames test exists for some N-heterocycles like quinoline and methylquinolines, while no effect was found for the tested isomeric compound isoquinoline (Nagao et al., 1977; Hollstein et al., 1978; Siderpoloulos and Specht, 1984; Takahashi et al., 1988; Saeki et al., 1996; Zeiger et al., 1992). The hydroxylated compounds 2(1H)-quinolinone and 1(2H)-isoquinolinone, which are mentioned as metabolites, showed no mutagenic effect in the Ames test (Willems et al., 1992). No data on genotoxicity or mutagenicity of the further reduced species 3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone are available.

The 3-ring N-heterocycles carbazole, acridine and phenanthridine were found to be mutagenic (IARC, 1999; Seixas et al., 1982; Dutson et al., 1997).

S- and O-heterocycles are rarely tested towards toxicity. Out of few data the 2-ring O-heterocycle benzofuran is mentioned as possibly carcinogen to humans (IARC, 1995). The U.S. EPA classified the 3-ring analog dibenzofuran as a compound with limited evidence of

animal carcinogenicity and absence of any data on human carcinogenicity (U.S. EPA, 1987). No data are present about toxicology of the S-heterocycles e.g. benzothiophene and dibenzothiophene.

Only few studies have focussed on ecotoxicity of heterocycles, while mostly azaarenes were investigated. Hartnik et al. (2007) studied the ecotoxicity of a tar oil contaminated groundwater. The group of N-heterocycles was found of relevance shown in the Microtox bioassay with *Vibrio fischeri*. When analysing only the fraction of PAHs, the potential risk of creosote-contaminated groundwater was found to be underestimated, because about 80 % of the total toxicity could not be explained by the presence of PAHs. The fraction primary containing quinoline compounds was responsible for 26 % of the total toxicity in the Microtox bioassay.

Bleeker et al. (1999) found an increase in acute toxicity towards the midge *Chironomus riparius* with an increasing number of the aromatic rings of 2-4 ring N-heterocycles. With the addition of one benzene ring for N- and S-heterocycles as well as PAHs, Eastmond et al. (1984) found an increase in toxicity of approximately one order of magnitude in the *Daphnia magna* immobilisation test. The S-heterocycles were generally found more toxic than their structurally similar PAHs.

There exist only few studies comparing the toxicity of the parent compounds with toxicity of microbially formed metabolites. However, data of performed studies showed that it is highly important to include these metabolites into the studies. Mueller et al. (1991) investigated the ability of microorganisms to degrade tar oil contaminants from groundwater. Most contaminants were removed, while only a slight decrease in toxicity was found. This lack in correlation was explained by the formation of non-detectable metabolites. Bleeker et al. (1999) studied the genotoxic effect in the Mutatox^{TM} test and toxicological potential on midge *Chironomus riparius* of the three-ring N-heterocycles acridine and phenanthridine as well as their metabolites. They reported that the transformation of these two substances to 9(10H)-acridinone and 6(5H)-phenanthridinone, respectively, strongly decreased ecotoxicity, while genotoxicity of 9(10H)-acridinone exceeded all compounds tested. The inhibition of nitrification by quinolines and their hydroxylated analogs was analysed in the MINNTOX-Test by Johansen et al. (1997a). Results showed that the toxicity remained when the quinolines were hydroxylated, while some hydroxylated quinolines even exhibited a higher toxicity potential than the parent compounds.

These data indicate that it seems of high relevance to evaluate the toxicological effects of NSO-heterocyclic compounds. Furthermore it is of high importance to obtain knowledge about biodegradability of heterocyclic compounds and to include their metabolites into the research activities.

1.5 Natural attenuation processes as a remediation strategy

Contaminated sites were often treated by active clean up-processes. However, some shortcomings of these strategies are known: Excavation often destroys the native ecosystems. It often may expose workers and nearby residents to elevated levels of contaminants. The pump-and-treat approach often cannot remove all contamination from a site (NRC, 1994). In addition, these techniques are very expensive and energy-consuming and in a number of cases they failed to reach the goal or only shifted the problem to other places.

Therefore, the passive approach called “natural attenuation”, which is discussed and used in the United States since the middle of the 90s, gained more interest, as a cost-effective, risk-based, low-intensity technology.

An acceptance of natural attenuation as a remediation strategy by U.S. EPA is only given if long-term monitoring of intrinsic processes termed "monitored natural attenuation" (MNA) is present. The definition of monitored natural attenuation in the U.S. EPA (1997) is the following:

"The term monitored natural attenuation, as used in this Directive, refers to the reliance on natural attenuation processes (within the context of a carefully controlled and monitored site clean up approach) to achieve site-specific remedial 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 favourable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentrations of contaminants in soil and groundwater. These *in situ* processes include biodegradation, dispersion, dilution, sorption, volatilization, and chemical or biological stabilization, transformation, or destruction of contaminants."

The only real destructive processes, which may lead to the complete breakdown of organic contaminants into harmless end-products, are biodegradation and abiotic degradation. Only due to these parameters a long-term efficiency can be established and the remediation goal can be reached.

MNA as sole decontamination strategy may be acceptable, if the prerequisite at the field site are fulfilled and active strategies are estimated to be disproportional. Therefore, always a site-specific decision is necessary if NA is a possibility. MNA is only an appropriate remediation method if its use will be protective of human health and the environment. Source control and performance monitoring are fundamental components of any MNA remedy for the controlling of present processes. MNA can be used as stand-alone technique as well as follow-up remediation. If active decontamination strategies as sole strategy are acceptable, the use of an MNA-concept is out of discussion.

However, until now natural attenuation is not accepted as a decontamination strategy according to § 2 Abs. 7 BBodSchG in Germany. The BBodSchG stated that groundwater and soil at contaminated sites have to be excavated or the expansion of the plume of contamination has to be prevented, without resulting risk, extensive annoyance or disadvantages for the individual or general public. Therefore, until now only active clean up-processes are accepted, while the way of implementation of natural attenuation processes into German law is advanced in discussion (LABO, 2005; KORA, 2005).

Evidence for natural attenuation

As prerequisite for the usage of natural attenuation as decontamination strategy, there has to be knowledge about the processes of transport and of distribution as well as metabolism of contaminants.
Therefore, the following chemical and geochemical parameters (Figure 3) may indicate the presence of natural attenuation processes:

- Decrease in concentration of contaminants along the contamination plume (best case: usage of a historical database showing stabilisation of the plume and/or loss of contaminant mass over time)
- Depletion of electron acceptors and formation of reduced species of the contaminants along the plume as the result (Wiedemeier et al., 1999)

- Formation of metabolic endproducts (carbon dioxide, methane, sulfide, nitrite, Fe(II), hydrogen), and, respectively, decreased concentration of organic compound as well as change of pH-value and lowered redox potential as the result of metabolism
- Formation of transformation as well as dead-end products due to microbial degradation (i. e. vinyl chloride, naphthoic acids) (Wiedemeier et al., 1999)
- Fractionation of isotopes of contaminants and electron acceptors along the plume
- Changes in enantiomeric ratio of chiralic compounds (Hunkeler et al., 1999, Zipper et al., 1998, Williams et al., 2003)

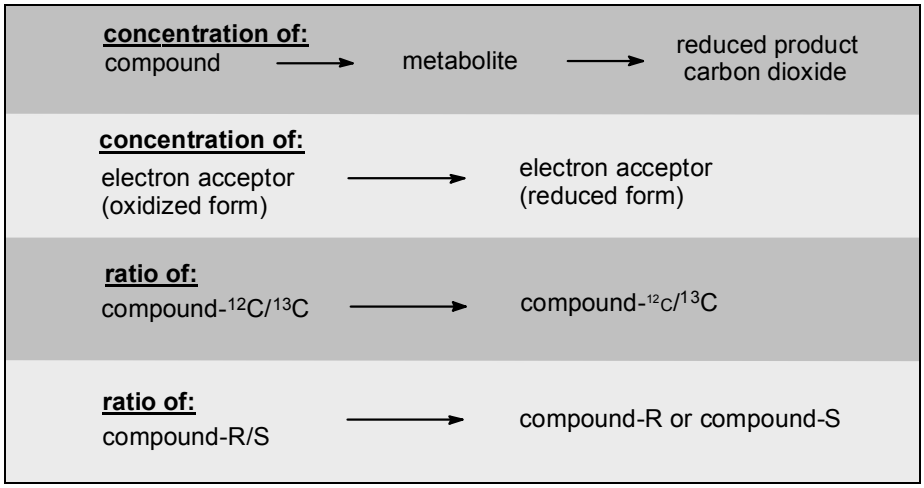


Figure 3: Parameters indicating the presence of natural attenuation processes.

The following data may be used to demonstrate the efficacy of natural attenuation:

1. Analyses of spatial and historical trends

A detailed map of the contamination plume can show the reduction in mass of dissolved contaminants. Correlation of contaminant concentration with conservative tracer (like chloride) can be used to show reduction in mass, apart from dilution processes and seepage velocity. Hence, it is not possible to differentiate microbial degradation from processes like dispersion as well as sorption. Using historical contaminant data, it is possible to demonstrate that the contaminant plume is shrinking, stable, or growing.

2. Demonstration of field-scaled indications for biodegradation

The demonstration of field-scaled biodegradation is based on *in situ* indicators. The consumption of electron acceptors, the formation of reduced species in the plume of contamination in correspondence to the disappearance of the contaminants or the occurrence of degradation products can be used as indication.

High direct evidence of degradation can be the formation of metabolic products. But metabolites can only be used to document degradation of the parent compounds if they are sufficiently specific. An ideal indicator of *in situ* biodegradation is a compound that could definitively be related to the metabolism of a specific compound. In addition, the compound should be an intermediate of mineralization rather than a dead-end product (Beller, 2000).

Furthermore novel techniques like changes in isotopic ratio or enantiomeric fraction may give information about microbial degradation (Meckenstock et al., 2004; Schmidt et al., 2004;

Müller and Kohler, 2004; Hunkeler et al., 1999; Zipper et al., 1998; Williams et al., 2003). However, these studies are only successful if there exists a fractionating enzyme, which is involved in the degradative process. Furthermore, carbon isotope fractionation is limited for small molecules. For compounds with large number of carbon atoms in the molecule the measured isotope fraction is "diluted".

3. Microbial evidence

Microbiological laboratory or field data can be used to show the presence of microbial populations capable to degrade the contaminants present at the site and/or the presence of activity in the plume.

For some contaminants like BTEX, PER or TRI it is possible to use some recent molecular techniques. These techniques include the detection of some specific genes which are coding for enzymes associated with particular degradation pathways. Additionally, the induction of degradative enzymes due to formed mRNA using PCR or the direct identification of specific microorganisms by their genetic features is possible (Davis et al., 2002; Stapleton et al., 1998).

1.6 Biodegradation of tar oil components

Degradation pathways are described for approximately 1000 compounds until now, while more than 10 million organic compounds are thought to be biodegradable (Wackett and Ellis, 1990). However, while most knowledge exists about the degradation of compounds in isolated pure cultures or enrichment cultures, the fate of compounds at tar oil contaminated sites is more complex and influenced by physical, chemical as well as physiological factors. Therefore, factors influencing the bioavailability and biodegradation as well as biotransformation have to be considered (Fetzner et al., 1998). In addition to geo-physical parameters like temperature, pH, moisture, salinity, oxygen concentration, availability of electron donors and acceptors as well as nutrients, and sorption of chemicals to particulate material have to be included into considerations. The level of concentration of chemicals may lead to an inhibition of biodegradation or mutual inhibition of contaminants (Bouchez et al., 1995; Wackett and Ellis, 1999). For some compounds only cometabolism occurs. This means that metabolism occurs only collectively and sequentially by different bacterial strains under certain environmental conditions. In such communities, the microbial partners share the resulting energy and exchange metabolites and reduction equivalents between each other.

Much information is available on the pathways involved in the aerobic degradation of aromatic hydrocarbons. Aerobic degradation leads to hydroxylated products due to the incorporation of one or two oxygen atoms from molecular oxygen by mono- or dioxygenase (Cerniglia, 1984; Gibson and Subramanian, 1984; Cerniglia, 1992). Anaerobic degradation was neglected for a long time, but in the beginning of the 1980s Kuhn et al. (1985) showed the existence of anaerobic microbial degradation.

The plume of most tar oil contaminated sites (and especially of the three sites investigated in this study) is characterized by limited oxygen supply. The respiration of carbon sources present leads to a depletion of oxygen and the formation of anoxic conditions. Therefore, degradation of aromatic contaminants is massively retarded.

In the following paragraph information on the anaerobic degradation of some PAHs, BTEX and heterocycles is given. In Table 2 data on the biodegradation under different redox conditions of compounds investigated in this study are summarized, mineralization as well as cometabolic transformation are included.

1.6.1 Anaerobic degradation of monocyclic and polycyclic aromatic compounds

Most knowledge exists about anaerobic microbial degradation of BTEX and several PAHs. For these compounds (toluene, *m*- and *o*-xylene, ethylbenzene, naphthalene, 2-methylnaphthalene) a general step in the degradation pathways is the addition of an unsaturated dicarboxylic acid, i.e. fumarate to the methyl group, resulting in the formation of benzylsuccinate analogs (Dolfing et al., 1990; Rabus and Widdel, 1995; Hess et al., 1997; Harms et al., 1999; Morasch et al., 2004; Kniemeyer et al., 2003; Biegert et al., 1996; Beller and Spormann, 1997a,b). The poorly reactive methyl carbon atom is converted to a methylene carbon in β−position to a carboxylic group, which allows the subsequent oxidation. For naphthalene and 2-methylnaphthalene the further degradation is characterized by a ring-reduction resulting in tetrahydro-, octahydro- and decahydro-2-naphthoic acid. The detection of naphthoic acid as well as their reduced species in the groundwater of tar oil contaminated sites shows the occurrence of these processes in the ground (Griebler et al., 2004; Gieg and Suflita, 2002). Furthermore, the formation of carboxylic acids of indan, indene, acenaphthene, and acenaphthylene in the naphthalene and 2-methylnaphthalene degrading pure culture was found as a cometabolic transformation step (Safinowski et al., 2006).

1.6.2 Anaerobic degradation of N-Heterocycles

In comparison to PAHs and BTEX, there exists only scarce knowledge about the anaerobic microbial degradability of NSO-heterocyclic compounds. To date there is only one pure microorganism available degrading N-heterocycles: *Desulfobacterium indolicum* (Bak and Widdel, 1986). Therefore, the knowledge about anaerobic degradation pathways of heterocycles is limited.

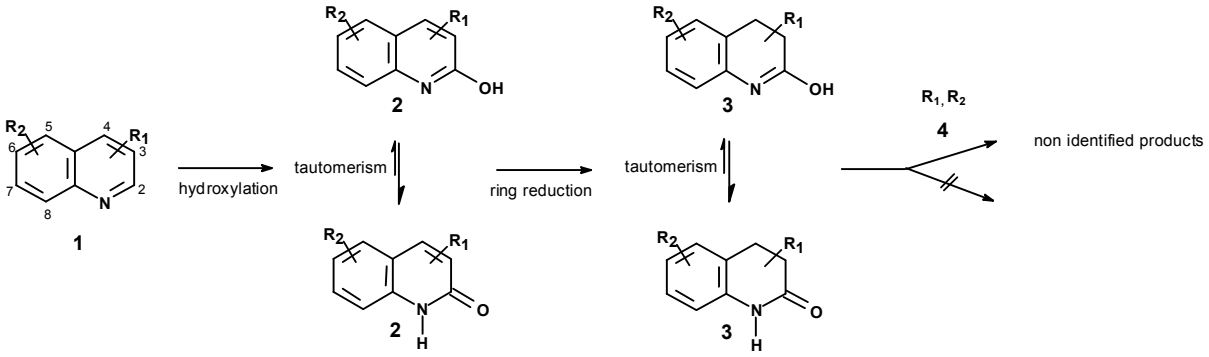
Studies investigating the aerobic as well as anaerobic degradation of heterocycles showed that S-heterocycles were less degradable than N- and O-heterocycles (Kuhn and Suflita, 1989; Meyer and Steinhart, 2000). Generally, the initial transformation of heterocyclic compounds seems to be often initiated by the incorporation of oxygen into the heterocyclic ring (Radledge, 1994; Pereira et al., 1987).

The microbial degradation of the two-ring N-heterocycle quinoline and its analogs was studied with microorganisms from different origin under several redox conditions, i. e. methanogenic as well as sulfate-, nitrate-, manganese-, and iron-reducing conditions (Bak et al., 1986; Bollag et al., 1991; Shanker and Bollag, 1990; Liu et al., 1994a,b, 1996). The initial step of degradation of quinoline and methylquinoline compounds is a hydroxylation in position 2, resulting in the formation of the 2-quinolinol compound, the enol form which exists in tautomeric equilibrium to the lactam compound 2(1H)-quinolinone (Figure 4). Using $H_2{}^{18}O$ Pereira et al. (1988) showed that the incorporated oxygen derives from water.

In studies with the pure culture of *Desulfobacterium indolicum* under sulfate-reducing conditions as well as with a biofilm system under denitrifying conditions, a second metabolite of quinoline, 3,4-dihydro-2(1H)-quinolinone, was identified, which was transformed further to unknown products.

Since the initial attack occurs at position 2, no transformation of 2-methylquinoline and dimethylquinolines with a methyl substituent at C_2 was observed (Shanker and Bollag, 1990; Johansen et al., 1997a,c; Pereira et al., 1987). The other studied methylated quinoline isomers, 3-, 4-, 6- and 8-methylquinoline, were transformed in analogy to the degradation of quinoline into their methyl-2(1H)-quinolinone compounds under denitrifying as well as sulfate-reducing conditions. Only 6-methyl- and 8-methyl-2(1H)-quinolinone were further reduced in minor amounts, resulting in 6-methyl- and 8-methyl-3,4-dihydro-2(1H)-quinolinone which persisted

(Johansen et al., 1997a, c). Under methanogenic conditions Pereira et al. (1987) found N- and O-methylations of quinoline analogs.



1	2	3	4
R_1, R_2=H	R_1, R_2=H	R_1, R_2=H	R_1, R_2=H
R_1=3-CH_3, R_2=H	R_1=3-CH_3, R_2=H		
R_1=4-CH_3, R_2=H	R_1=4-CH_3, R_2=H		
R_1=H, R_2=6-CH_3	R_1=H, R_2=6-CH_3	R_1=H, R_2=6-CH_3	
R_1=H, R_2=8-CH_3	R_1=H, R_2=8-CH_3	R_1=H, R_2=8-CH_3	

Figure 4: Proposed degradation pathway of quinoline and methylquinoline compounds, identified under sulfate-reducing conditions with *D. indolicum* and under denitrifying conditions with a mixed culture from a waste water treatment plant (Johansen et al., 1997a, c). R_1 and R_2 represent the respective substituents in both rings.

There is less information available about the anaerobic degradation of isoquinoline. In analogy to anaerobic quinoline degradation, the first step in isoquinoline transformation is a hydroxylation next to the N-heteroatom, resulting in 1(2H)-isoquinolinone (Johansen et al., 1997a; Pereira et al., 1987), which was found under denitrifying as well as methanogenic conditions. Studying methanogenic transformation Pereira et al. (1987) found minor amounts of the N-methylderivative of isoquinoline.

The monocyclic compounds pyridine and methylpyridines are known to be degradable under several redox conditions (Kaiser and Bollag, 1991; Kuhn and Suflita, 1989; Bak and Widdel, 1986; Ronen and Bollag, 1991, 1992; Battersby and Wilson, 1989; Kaiser et al., 1996). Minor knowledge exists about degradation pathways. Somehow, oxygenated metabolites might be formed.

Similarities to the degradative pathway of quinoline compounds were observed in the degradation of indole. Complete mineralization has been reported under denitrifying, sulfate-reducing as well as methanogenic conditions (Wang et al., 1984; Berry et al., 1987; Madsen and Bollag, 1989; Shanker and Bollag, 1990; Liu et al., 1994a; Licht et al., 1996).

As has been reported for quinoline compounds, also for indole the first step of degradation is a hydroxylation in position 2, forming 2-oxindole, followed by a second hydroxylation in position 3 where indole-2,3-dione is formed. This metabolite is subsequently degraded to anthranilic acid. These metabolites were found in the sulfate-reducing culture of *D. indolicum* (Johansen et al., 1997c) as well as in a denitrifying mixed culture from sewage sludge (Madsen and Bollag, 1988). Hydroxylation of methylated indole derivatives was only observed for 3- and 7-methylindole, while it was blocked in 1- and 2-methylindole. The metabolites formed, 3- and 7-methyloxindole, were not further degraded (Johansen et al., 1997c).

The degradation of the higher sorbing and therefore less bioavailable three-ring N-heterocycle carbazole is only described under methanogenic conditions, while no metabolites were identified until now (Pereira et al., 1987). In other studies carbazole was found to be non-biodegradable (Liu et al., 1994a; Dyreborg et al., 1997).

Transformation of acridine to 9(10H)-acridinone was assumed from metabolites occurring in the further degradative pathway (Knezovich, 1990), while the further degradation of 9(10H)-acridinone is not described until now. Furthermore, there exist no data about the degradation of phenanthridine and its hydroxylated analog 6(5H)-phenanthridinone.

1.6.3 Anaerobic degradation of S-Heterocycles

Until now, no pure cultures were isolated, which can use S-heterocycles like thiophene and benzothiophene as sole source of energy and carbon. However, the formation of H_2S has been detected during the anaerobic degradation of thiophene (Kurita et al., 1971; Kohler et al., 1984). Furthermore desulfurization was also found by Kim et al. (1990) and Marcelis et al. (2003). Studying a naphthalene degrading enrichment culture, the cometabolic transformation of benzothiophene leading to carboxybenzothiophene derivatives (2-carboxy-, 5-carboxy- and dihydrocarboxybenzothiophene) was found (Annweiler et al., 2001). The carboxylation is an non-specific reaction, including the attack of the benzene- as well as the thiophene-ring. Although the transformation in the laboratory studies was incomplete, the mentioned metabolites were found in groundwater showing the relevance of this transformation in the environment (Annweiler et al., 2001; Griebler et al., 2004; Safinowski et al., 2006). The cometabolic transformation of benzothiophene in a naphthalene and 2-methylnaphthalene degrading pure culture resulted in the formation of benzothiophene methylsuccinic acid and benzothiophene methylenesuccinic acid (Safinowski et al. 2006), which indicated the analogy to anaerobic degradation of a huge number of compounds as mentioned above.

1.6.4 Anaerobic degradation of O-Heterocycles

The monocyclic O-heterocycles furan and 2-methylfuran were found to be degraded in sulfate-reducing as well as methanogenic microcosms within 3-8 months, while no metabolites were detected (Kuhn and Suflita, 1989).

In microcosm studies with aquifer material from a field site, no degradation of benzofuran was found under nitrate- as well as sulfate-reducing conditions (Licht et al., 1996). However, Safinowski et al. (2006) found the cometabolic transformation of benzofuran in a naphthalene and methylnaphthalene degrading pure culture resulting in the formation of benzofuran-carboxylic acid.

Table 2: Data on degradation studies with hetero- and homocyclic compounds under anoxic conditions.

compound	transformation				references
	nitrate	sulfate	iron	methanogenic	
N-Heterocycles					
quinoline	+[a-d]	+[a,c-e]	+[w]	+[c, g, h]	[a] Liu et al., 1994a
isoquinoline	+[i]	-[b]		+[g]	[b] Johansen et al., 1997c
2-methylquinoline	-[i]	-[b,g, h]		-[g, h]	[c] Licht et al., 1996
4-methylquinoline	+[i]	+[b]		+[g ,h]	[d] Brockman et al., 1989
6-methylquinoline	+[i]	+[b]		+[h]	[e] Licht et al., 1997b
2(1H)-quinolinone	+[i]	-[a] / +[b]	+[w]	+[g, h]	[f] Dyreborg et al., 1997
1(2H)-isoquinolinone	-[i]			+[g, h]	[g] Pereira et al., 1987
3,4-dihydro-2(1H)-quinolinone	+[i]	+[b]			[h] Liu et al., 1994b
2,4-dimethylquinoline				-[h]	[i] Johansen et al., 1997a
2,6-dimethylquinoline				-[h]	[j] Knezovich et al., 1990
4-methyl-2(1H)-quinolinone	-[n]	-[b]		-[n]	[k] Safinowski et al., 2006
acridine	+[j]	+[j]		+[j] /-[g]	[l] Kim et al. 1990
9(10H)-acridinone					[m] Lizama et al., 1995
carbazole	-[a] / +[g]	-[a]		+[g] /-[a]	[n] Annweiler et al., 2002
6(5H)-phenanthridinone					[o] Meckenstock et al., 2000
S-Heterocycles					[p] Annweiler et al., 2001
benzothiophene	-[c,s]	+[l, k, p,s]/ -[c, f]		+[q] / -[c]	[q] Arvin et al., 1988
3-methylbenzothiophene					[r] Godsy, 1993
dibenzothiophene	-[v]	+[l, k]		+[q] / -[r]	[s] Mundt et al., 2003
O-Heterocycles					[t] Coates et al., 1997
benzofuran	-[c, v]	-[c,f] / +[k]		+[r] / -[c]	[u] Rockne et al., 2000
2-methylbenzofuran				-[r]	[v] Broholm et al., 1999
2,3-dimethylbenzofuran					[w] Liu and Kuo, 1996
dibenzofuran	+[s]	+[s]			[x] Ambrosoli et al., 2005
methyldibenzofuran					[y] Mihelcic and Luthy, 1988
PAHs/Homocycles					[z] Anderson et al., 1999
indan	+[s]	+[k,s]			[ä] Chang et al., 2001
indene	-[s]	+[k] / -[s]			
acenaphthene	+[y]	+[k]			
fluorene	+[x]	+[t]			
naphthalene	+[u]	+[o, n, t]	+[z]	+[ä]	
1-methylnaphthalene	-[s]	+[k] / -[s]			
2-methylnaphthalene	+[s]	+[n, s]			

Legend: + = decrease of compound determined; - = no decrease observed; a differentiation if the decrease is due to mineralization or cometabolic transformation was not done.

2. AIM OF THE THESIS

Natural attenuation at contaminated sites is defined as the reduction in toxicity, mass and/or mobility of a contaminant without human intervention. This includes biological as well as abiotic processes. But only destructive processes such as biodegradation should be considered as appropriate decontamination strategy. Former coke manufacture, gasworks as well as timber impregnation sites are contaminated by tar oils. Besides PAHs NSO-heterocyclic compounds are present at about 5-13 % in tar oils, which are strongly smelling and toxic compounds, but seldom analysed in the groundwater of these sites.

The assessment of the relevance of NSO-heterocycles in the groundwater of three tar oil contaminated sites was one of the aims of the thesis. An additional important point was to elucidate if the fate of NSO-heterocycles and their metabolites is applicable to demonstrate natural attenuation processes. Therefore, the following approaches were performed:

Analytical methods were developed for the determination of 1-3-ring heterocycles as well as their metabolites to monitor the groundwater of three sites for the presence of these compounds besides PAHs. Special emphasis was set on N-heterocyclic compounds. The relevance of heterocyclic compounds as pollutants should be evaluated with focus on the mass flow of the different types of contaminants within the contaminants plumes. Laboratory microcosms with the authentic contamination, on-site columns as well as a high-resolution multi-level well should be investigated to obtain indications for the presence of microbial degradation.

Studies on the suitability of metabolites of quinoline compounds as indicator for biological natural attenuation processes should be performed. Degradation of quinoline compounds in single, formation and elimination of metabolites of quinoline derivatives should be analysed in microcosms. In addition, the influence of microbial degradation of N-heterocyclic compounds on ecotoxic, mutagenic, and genotoxic potential of these compounds should be studied to assess an environmental risk due to contaminated groundwater. Another point of interest was the analysis of the parameters influencing distribution of quinoline compounds and their metabolites in a plume, such as sorption and dissolution from tar oils to the water phase. Finally, a concept using hydroxylated metabolites and parent compounds should be developed and tested on its feasibility to determine natural attenuation.

3. METHODS

3.1 Investigated tar oil contaminated sites

In the present study three tar oil contaminated sites, with different origin in contamination were analysed for the environmental importance of tar oil compounds. These sites allowed to analyse the group of heterocyclic compounds, a highly water soluble fraction from tar oils.

3.1.1 The Castrop-Rauxel site (former coke manufacture)

The former coal mine with coking plant is located in Castrop-Rauxel, North Rhine Westphalia, Germany (Figure 5).

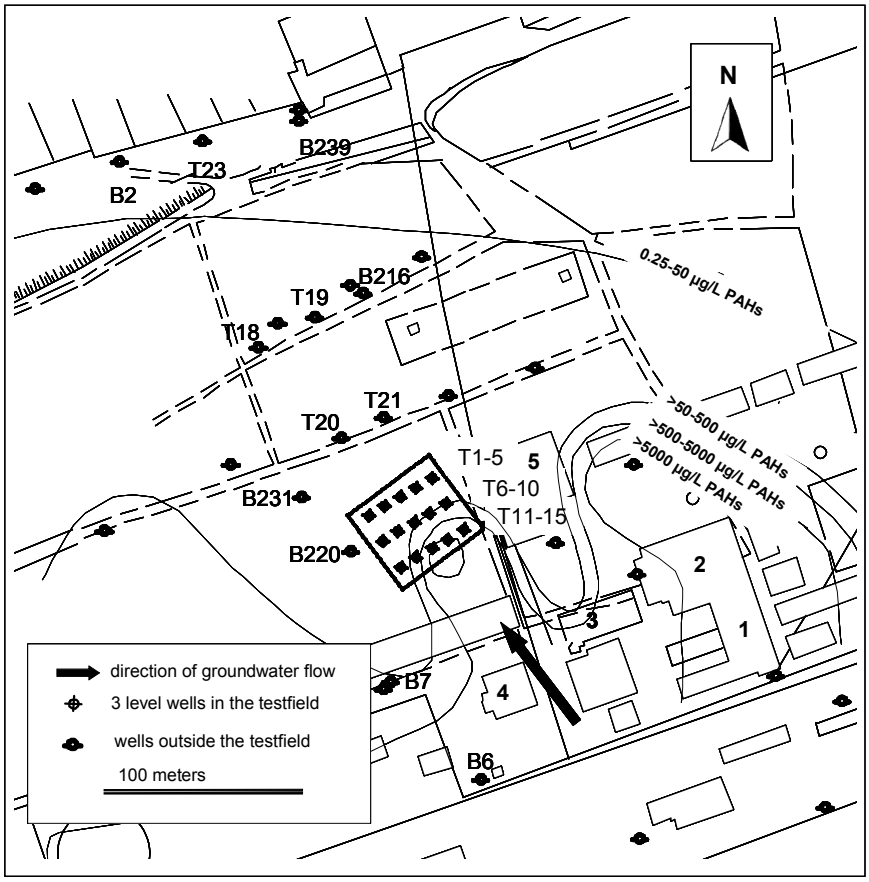


Figure 5: Schematic overview on the field site at Castrop-Rauxel. A testfield with 15 multi-level wells (4, 5, 7 m depth) is located in the "edge" of the contamination plume. T1-5; T6-10; T11-15 are the denotations of the 15 three-level wells. The former coal tar production facility was built of the following parts: (1) plant for condensation of naphthalene, (2) purification plant for benzene, (3) benzene plant, (4) plant for extinguish coke and purification, (5) plant for mixing coal and coke.

From the beginning of the 20th century to 1972 the site was used for the production of coke and various tar oil products (like benzene, naphthalene or tar distillate) (Schnier, 1999). Then the production factory was destroyed and the ground was filled with the existing building rubble. The contamination of groundwater in quaternary deposits as well as in marl rocks resulted from discharging waste into the ground and because of leakage and destruction during the Second World War. Real data about the date and points of contamination are scarce. The main sources of the contamination of the aquifer are the plant for extinguish coke and coke purification as well as the benzene plant in the southern part of the field site. In this study the contamination of the quaternary aquifer was investigated: The groundwater flow in

quaternary deposits is estimated to be 0.074–0.085 m/d confirmed by tracer experiments (Vooren, 2006). The monitoring of groundwater was repeated in intervals of several months since December 2003 and shows a flow of groundwater into the direction of West-Northwest. The resulting plume of PAHs is estimated to be of ~325 m in length, of 250 m in breadth and of 50 m in depth (Schnier, 1999).

The texture of the quaternary aquifer is mainly contributed by fine and medium sand (95 %), with some silty and gritty samples. The organic carbon content is <0.1 % and the clay mineral content approx. 0.25 %. Hydraulic conductivity (K_f-value) of the aquifer material is in the range of 3-25 × 10^{-5} m/s (Breul, 2004). The saturated thickness of this granular aquifer varies from < 5.0 to 7.4 m. At its base the aquifer is terminated by a silky layer of weathered marl.

Anaerobic conditions in the quaternary aquifer are indicated by low redox potential of groundwater and oxygen concentration below 0.5 mg/L. Sulfate seems to be the favourable electron acceptor due to the high concentrations in the groundwater (300-1300 mg/L) originating from leaching of the anthropogenic overlain filling. Although sulfide concentrations were lower than limit of detection in the groundwater samples, textile tapes (GAIASAFE GmbH, Marburg, Germany) were incubated in the wells and showed the presence of sulfate-reduction (Breul, 2004). In addition, iron might be used as electron acceptor shown by concentrations of 0.08–55 mg/L Fe(II) in the groundwater. Reduced iron may also lead to no detectable concentration of sulfide due to precipitation of iron-sulfide. The concentration of nitrate in the groundwater is lower than 1 mg/L all over the site and therefore nitrate-reducing conditions are of minor relevance. The pH-values of groundwater samples in the testfield are in the range of 6.6-7.2 with no clear spatial trend.

A set of wells, spread over the field site of 39 ha, was established to obtain detailed information on the distribution and fate of contaminants. An additional testfield, which spans an area of 36 × 40 m, with 15 three-level wells (depth approx. 4, 5 and 7 m), was installed in December 2003 for the in-depth analysis of the fate of contaminants in the quaternary aquifer. The three lines of five wells are approximately in parallel to the isopiestic lines.

3.1.2 The Düsseldorf site (former gasworks site)

The investigated former gasworks site is located in Düsseldorf-Flingern in the River Rhine valley, Germany. Over a period of at least 30 years the production of benzene, gas and coke resulted in the deposition of tar oil on an area of 170,000 m^2 resulting in a contaminant plume of about 600 m length and 100 m width (Eckert, 2001; Wisotzky and Eckert, 1997).

In several remediation activities starting in 1996 the major part of the tar oil residues was removed from the unsaturated source zone (7000 m^2, depth of 6 m), leading to a reduced size of the plume of about 200 × 40 m in dimension. The maximum concentration of BTEX decreased from originally approx. 100 mg/L to 20 mg/L on average, while concentrations of PAHs remained more or less constant at about ~10 mg/L (Eckert, 2001).

The contaminated quaternary aquifer is characterized by sand and gravel and shows a relatively homogeneous composition and permeability across a thickness of about 10 m. K_f-values are in the range of 2 × 10^{-5} to 2 × 10^{-3} with an average of 1 × 10^{-3} m/s. The aquifer is interrupted by thin gravel inclusions in deeper layers (>12 m). In about 16 m below land surface the aquifer is confined by low permeable tertiary fine sand layers. Groundwater in this section of the aquifer follows a hydraulic gradient of about 6 % from East towards West with a velocity of 0.5-2 m/d.

Sulfate as well as iron-reducing conditions were found to be present (Eckert, 2001; Wisotzky, 2000) and were clearly indicated by high concentrations of sulfide (1-13 mg/L) and dissolved

iron (0.1-19 mg/L). Sulfate concentrations were found within 5-200 mg/L. Nitrate was present in the range of 1-22 mg/L and is considered as non-relevant electron acceptor. The pH-value of groundwater samples is in the range of 6.8-7.6 (data of groundwater characterization were kindly provided by L. Richters, Stadtwerke Düsseldorf-Flingern).

In Figure 6 the distribution of the wells investigated in this study over the Düsseldorf-Flingern field site is shown, forming 5 lines downstream the source for the plume characterization.

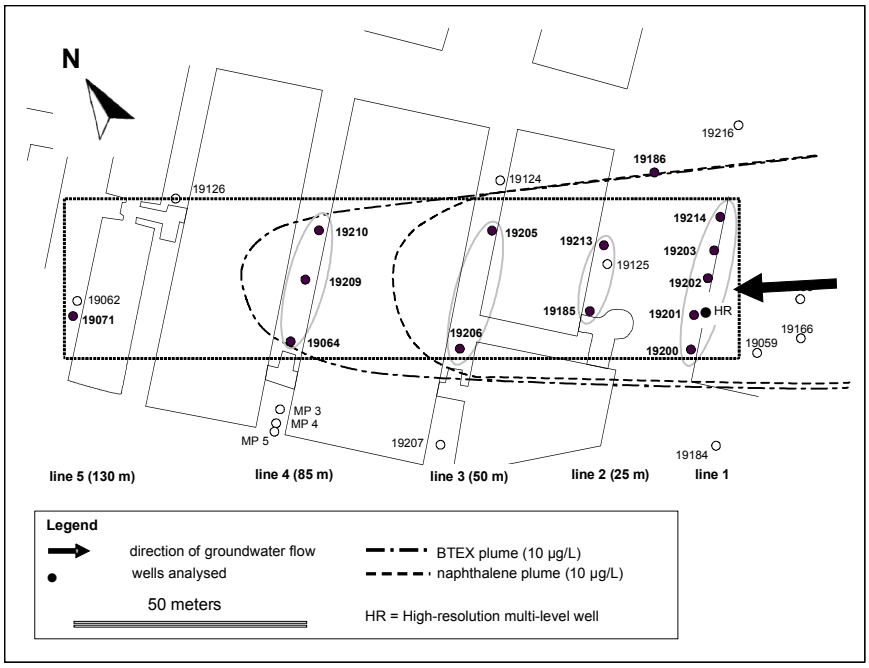


Figure 6: The Düsseldorf site with the distribution of the wells and plume of PAH and BTEX in July 2004.

In the highest contaminated region of the Düsseldorf-Flingern site, next to well 19201 a high-resolution well is located. The total length of the high-resolution well is about 12 m, while there were 12 single units assembled, each consisting of a HDPE tube of 1 m length and 15 cm outer diameter. At distances of either 3 cm (high-resolution), 10 cm (middle resolution) or 33 cm (low resolution) from each other, small filter units (sintered bronze silencers, Norgren, UK) were inserted (Figure 7). A stainless steel capillary (Ø ID 1 mm) inserted tightly through a rubber stopper into the filter port connects the filter with the surface (Figure 7; for a detailed description see Anneser et al., 2007).

The installation of the well was performed in June 2005 using hollow-stem auger drilling. The well was introduced followed by the removal of the supporting steel pipes. The sediment was allowed to collapse, no artificial filter sands were added leading to a perfect embedding of the filter ports in the aquifer sediments. Therefore, hydraulic shortcuts from artificial sands was prevented. The aquifer material showed no hints for the presence of tar oil phase. It was characterized by the following grain size distribution: 0.2-37.9 % grit, 14.8-82.5 % medium sand, 2.8-72.4 % fine sand, 0-3.3 % coarse silt, 0-3.6 % medium silt, 0-3.3 % fine silt and 0-5.5 % clay particles. K_f-values were found within 1.89×10^{-5} and 1.54×10^{-4} m/s. The total organic matter was found within 0.14-0.87 % (data were kindly provided from B. Anneser and C. Griebler, GSF, Neuherberg, Germany).

Samples from high-resolution well were taken in August 2006 and February 2007. For sampling, the selected steel capillaries were connected to multi-channel peristaltic pumps (IPC-N or IPC) using 2-stopper Fluran HCA tubes (Ismatec, Wertheim, Germany). A volume of 100 mL of groundwater sample of selected depth was taken at a rate of about 2 mL/min.

Samples were acidified to pH 2 by the addition of HCl. Sample preparation was done according to chapter 3.2 using SPE-enrichment, in contrast, here the factor of enrichment was only of about 10.

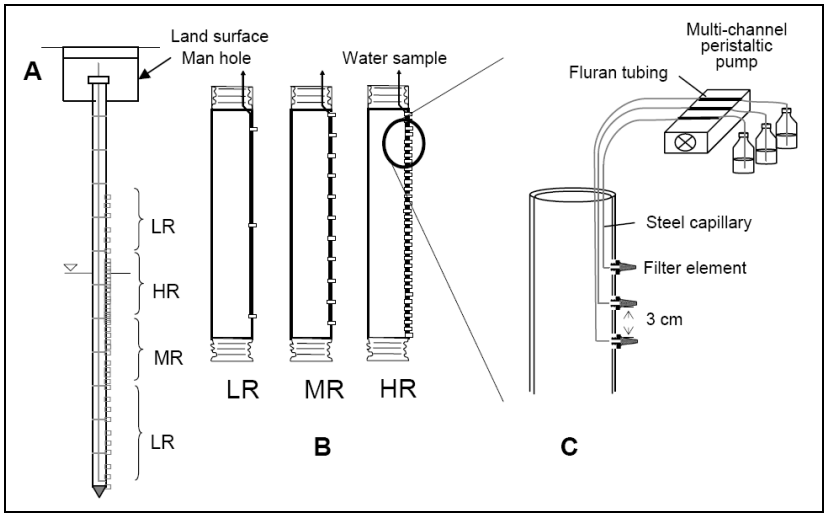


Figure 7: (A) Construction of the high-resolution multi-level well; (B) different modules: LR = low resolution (filter screen intervals of 30 cm); MR = middle resolution (10 cm filter intervals); HR = high-resolution (3 cm filter intervals); (C) View on filter screens and sampling equipment assembly (Anneser et al., 2007).

3.1.3 The Wülknitz site (former wood-preserving facility)

The former and today's wood impregnation site is located in Wülknitz, Saxony, Germany. Since 1875 the site was used for the preservation of wood by salt and tar oils. Due to leakage and losses during treatment the main contamination is located in the region of the impregnation hall, the area of delivery of tar oils and storage of tar oils as well as the area of draining the treated timbers. While reclamation of the non-saturated zone has taken place in 2000, the quaternary aquifer remained contaminated with tar products. In Figure 8 the distribution of wells over the field site is shown.

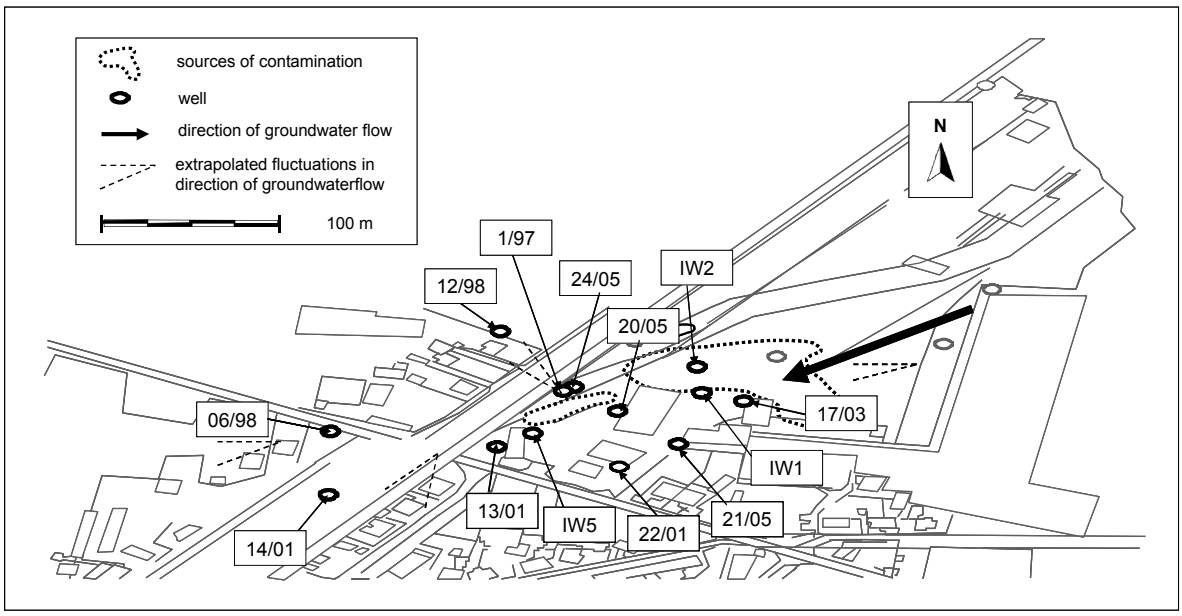


Figure 8: Overview of the timber impregnation site at Wülknitz. Numbers mark the location of wells.

The quaternary aquifer is characterized by a thickness of 30 m and a sandy and gritty structure, which is completed by tertiary clay and silt at the basement. Total organic carbon is found between 0.2-0.3 %. The groundwater flow is about 0.19-0.25 m/d, generally from North-East to South-West, while there are determined high fluctuations in direction of groundwater. The hydraulic conductivity (K_f-value) of the aquifer material was found about 2×10^{-3} m/s. An area of 3,000 m^2 is contaminated with a contamination plume of approx. 300 m length. In the source region contamination was found up to a depth of 17 m. The major redox conditions are discussed as sulfate- as well as iron-reducing conditions (Leibenath et al., 2006). Concentrations of sulfate were 50-370 mg/L, dissolved iron(II) 0.1-27 mg/L. But also nitrate was found in concentrations of 3-70 mg/L. pH-values in the groundwater were found all over the site in the range of 6.7-7.2, while no tendency with depth or direction of groundwater flow was evident.

3.2 Sampling and preparation of groundwater samples

The groundwater samples were generally taken with a flow of ~3 L/min. Each well was flowed through for 20 min prior to sampling. The field parameters pH, temperature, redox potential, electric conductivity and dissolved oxygen were monitored during sampling by portable instruments. Sampling was carried out after the stabilisation of the field parameters.

The groundwater samples were generally collected and stored in 1 L-glass bottles, acidified with hydrochloric acid to pH ~2 and closed with Teflon-coated caps to avoid sorption and volatilization.

Groundwater samples of the testfield of the Castrop-Rauxel site were taken in March 2005. The vertical resolution of the 3-level-wells was about 1 m, in depths of 4, 5, and 7 m. Further samples were taken from downstream area (T18, T19). Samples for headspace analyses were taken in December 2005. For the analyses of the plume in Düsseldorf-Flingern groundwater from wells distributed over the site was taken in October 2005. The vertical resolution of wells was about 0.5-1 m in depths between 6.5-12 m. The Wülknitz field site was investigated in July 2005 as well as December 2005. In most cases three different depths were analysed, while the vertical resolution of wells was about 2-4 m.

In the laboratory 1 L of groundwater was adjusted to pH 4, spiked with internal standards (100 µL D_7-quinoline (10 mg/L) in methanol for HPLC-MS-MS- and 250 µL 1,1´-binaphthyl (200 mg/L) for HPLC-DAD-measurements and extracted using polymeric SPE material (200 mg of hydroxylated polystyrene-divinylbenzene copolymer, surface area 1000 m^2, average particle diameter 90 µm) (Isolute ENV$^+$, Separtis, Grenzach-Wyhlen, Germany). No conditioning of the cartridges was necessary. Enrichment of the groundwater sample was performed using low water-jet vacuum with approx. 3-4 droplets/sec. (~ 10 mL/min). Elution was carried out with 10 mL acetone/methanol (50/50; v/v) without vacuum adding bit by bit 2 mL of the solvent mixture. The eluate was collected in a graduated vessel and mixed before the analyses. These extracts were used for the measurements of HPLC-DAD and HPLC-MS-MS. To check contaminations due to the sample preparation a blank-control using one litre of Millipore water was enriched and eluted in the same way. Parameters of the method validation are described in Mundt and Hollender (2005).

To reach lower limits of detection 10 L groundwater sample of well 19071 in Düsseldorf-Flingern were enriched using 4 SPE-cartridges and eluting them together with 10 mL of the acetone/methanol mixture.

3.3 Analytical Methods

3.3.1 HPLC-DAD method for tar oil compounds

For the analyses the method of Mundt and Hollender (2005) was used. Chemicals of highest purity were used (see chapter 7.1, appendix). Only small modifications of the analytical gradient and the spectrum of analysed compounds were done. The liquid chromatography was carried out with an LC system HP1100 (Agilent®, Waldbronn, Germany) with UV-diode array detector. Separation was achieved on a RP-column SynergiTM HydroRP C18 (250 mm × 2 mm I.D., 4 µm particle size, Phenomenex®, Aschaffenburg, Germany) by gradient elution. The gradient with acetonitrile and phosphate buffer pH 7 (10 mM) was as follows for the analyses of groundwater samples: 20 % acetonitrile for 2 min, up to 25 % acetonitrile in 2 min, in 10 min up to 50 %, in 10 min up to 60 %, in 12 min up to 75 % and in 7 min up to 100 % acetonitrile. Within 5 min the gradient set down to 20 % acetonitrile and remained for 6 min for equilibration.

Depending on the polarity of the investigated single compounds in quinoline microcosms, sorption experiments, tests on ecotoxicity and determination of water solubility, two different gradients were used: for hydroxylated quinoline compounds the gradient started with 20 % acetonitrile for 2 min, between 2 and 14 min acetonitrile was raised up to 50 %, then between 14 min to 20 min up to 60 %, between 20 to 23 min decreased to 20 % acetonitrile followed by equilibration until 30 min; for quinoline, isoquinoline, and methylquinolines the gradient started with 50 % acetonitrile for the first 2 min, between 2 and 6 min acetonitrile was raised up to 60 %, until 18 min up to 75 %, between 18 and 25 min raised to 100 % and finally decreased to 50 % acetonitrile until 30 min, followed by equilibration until 36 min.

A flow rate of 0.2 mL/min and a column temperature of 25 °C was adjusted. The injection volume was 10 µL. The diode array detector was set up to detection wavelength of λ = 210 nm (and λ = 254 nm for single compounds acridine and 9(10H)-acridinone). Spectra acquisition was performed from λ = 190 nm up to λ = 400 nm. Limits of detection are presented in Table 23, appendix. Quantification was performed by external calibration curves. 1,1´-Binaphthyl was only used as control of SPE-enrichment.

3.3.2 HPLC-MS-MS method for N-heterocyclic compounds

Chemicals of highest purity available were used; detailed information are given in chapter 7.1, appendix.

For the analyses of groundwater samples an LC system HP1100 (Agilent®, Waldbronn, Germany) with MS-MS (API 3000 as well as API 4000, Applied Biosystems MDS SCIEX, Foster City, U.S.A.) was used. Separations were carried out on a RP-column SynergiTM Hydro RP C18 (250 mm x 2 mm I.D., 4 µm particle size, Phenomenex®, Aschaffenburg, Germany) using a gradient program. As mobile phase ammonium acetate buffer (2 mM, pH 7) and acetonitrile with the following gradient was used: 2 min at 20 % acetonitrile, 2 to 22 min up to 75 %, 22 min to 27 min up to 75 %, 27 to 30 min up to 100 % acetonitrile and hold for 4 min, 34 to 45 min back to 20 % acetonitrile and equilibration. The flow rate was 0.2 mL/min and column temperature used was 32 °C. LC-MS-MS analyses were performed in multiple reaction mode (MRM) with positive electrospray ionisation. Spray voltage was about +5000 V and temperature at 390 °C. Nitrogen was used as sheath gas, auxiliary gas as well as collision gas. Protonated molecular ions were used as precursor ions $[M+H]^+$. The monitored transitions, limits of detection and quantification as well as further parameters of method validation determined with API3000 are presented in chapter 4.1. Generally sensitivity of API4000 was about a factor of ~10 higher.

Stock solutions of 2 g/L in methanol were used to prepare a standard mixture of 100 mg/L. Calibration standards in acetone/methanol (50/50; v/v) were diluted resulting in concentrations in the range of 1 µg/L-1 mg/L (1, 2, 5, 10, 25, 50, 75, 100, 200, 400, 600, 800, 1000 µg/L). An external calibration curve plotting peak areas versus concentration was obtained including at least 10 calibration points. A standard curve was acquired at the beginning as well as at the end of the series of measurements. In between, several standards were measured as control of the sensitivity of the instrument. D_7-quinoline was only used as control of the SPE-enrichment as well as marker of retention time.

3.3.3 LC-NMR and Flow Injection-MS methods

The analysed sample originated from a depth of 5 m (well T13) in the middle of the contamination plume in Castrop-Rauxel. Analyses were performed by M. Elend and A. Preiß (Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany).

The used HPLC system consisted of a Bruker Saxonia LC-22 pump (Bruker, Leipzig, Germany), a Rheodyne 7725i injection valve with a loop of 50 µL (Cotati, USA), a Bischoff Lambda 1010 UV detector (Bischoff, Leonberg, Germany) and a Bruker BPSU-36 peak sampling unit. The NMR spectrometer was a Bruker Avance DRX 600 (Bruker BioSpin, Rheinstetten, Germany) equipped with a 4-mm z-gradient flow probe ($^1H/^{13}C$ inverse, active detection volume of 120 µL).

The LC-NMR runs were carried out on a Merck RP 18e column (4.0 x 250 mm, 5 µm). Solvent A was acetonitrile, solvent B a mixture of acetonitrile (30 %) and 1 g/L trifluoroacetic acid-D_1 in D_2O (70 %). The runs started with 1 % A, keeping the composition of the mobile phase for 40 min. The percentage of A was increased within 140 min to 29 %, then within 60 min to 50 % and finally within further 120 min to 57 % of acetonitrile. The flow rate was 0.1 mL/min and a volume of 50 µL of the sample was injected. The ^1H NMR data were acquired using the Bruker lc2wetdcus sequence for multiple solvent suppression. Free induction decays (FIDs) were collected into 16 K data points (sweep width 9615.4 Hz) with a relaxation delay of 0.8 s and a flip angle of 90° (9.7 µs). The NMR chromatogram was recorded within 250 rows, each row consisting of 48 FIDs. As far as chemical shifts data were extracted from the NMR chromatogram, they were referenced to acetonitrile (^1H, $\delta = 2.00$ ppm).

In order to confirm the structure, compounds identified *on line* by LC-NMR were isolated by cutting the corresponding peak in several HPLC runs. The HPLC separation was carried out on a Merck RP 18e column (4.0 x 250 mm, 5 µm) using the following mobile phase: solvent A was acetonitrile, solvent B was a solution of 0.2 % formic acid in H_2O. The gradient started with 20 % A, and this composition was maintained for 2 min. Solvent A was stepwise increased within 30 min to 75 %. The flow rate was 1.0 mL/min and compounds were detected at 254 nm. For further NMR measurements, the combined HPLC cuts of each peak were evaporated to dryness in a gentle nitrogen stream, re-dissolved in 55-60 µL of $CDCl_3$/DMSO-D_6 (50/50; v/v) and filled into NMR microtubes for FI-MS measurements. The HPLC cuts were used directly. The *off line* NMR measurements were carried out with a 2.5 mm $^1H/^{13}C$ inverse dual probe head with z-gradient. ^1H NMR spectra were acquired with the standard Bruker pulse sequence zg 30 and referenced to DMSO-D_6 (^1H, $\delta = 2.51$ ppm): sweep width 7788.162 Hz, 32 K data points, 2.4 sec acquisition delay, 64-1024 scans.

MS measurements (ESI positive) of samples obtained from HPLC cuts were carried out in the flow injection mode on a Bruker Esquire-LC mass spectrometer. Typical conditions were as follows: flow: 400 µL/h; skim voltage was varied between 7.9 and 30.8 V; dry gas: 5 L/min

nitrogen at 300 °C; nebulizing gas: nitrogen 11 psi; scan range 40 to 800 m/z; 16 averages per spectrum.

3.3.4 Identification of metabolites using high-resolution MS

High-resolution-MS was performed using the combined LTQ-Orbitrap™ mass analyser (Thermo, Waltham, MA, USA). The HPLC system consisted of a Flux Instruments Rheos 2200 pump (Flux Instruments AG, Basel, Switzerland) and a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). Chromatographic analysis was performed using acetonitrile and ammonium acetate buffer (2 mM; pH 7) with an RP-column Synergi™ Hydro RP C_{18} (250 mm x 2 mm I.D.; 4 µm particle size; Phenomenex®, Aschaffenburg, Germany). A flow rate of 0.2 mL/min at room temperature was used. Gradients were the same as presented in chapter 3.3.1 and 3.3.2. Ionisation was done in the positive mode of electrospray with a source voltage of 3.5 V, capillary temperature of 350°C, and capillary voltage of 35 V.

Metabolites were identified using accurate full scan mode at a resolving power of 100,000 and phthalate ion as lock mass for internal calibration. Processing of data was done using Xcalibur Qual Browser (Thermo Electron, San Jose, CA). Parent masses of identified metabolites were subjected to chemical formula calculator. Reported errors of mass measurement were always smaller than 1 ppm. Further confirmation for distinct identification was achieved by a numerical isotopic pattern match.

3.3.5 Headspace-GC-MS method for volatile tar oil compounds

A GC-MS-system HP 6890 Series GC System, MS 5973 Mass Selective detector (Agilent Technologies, Böblingen, Germany) equipped with headspace autosampler (Tekmar™ 7000, Teledyne Instruments, Magdala, Germany) was used for the analyses.

The parameters of the autosampler were as follows: loop 1 mL; pressure generation in the vial: 100 kPa; plate temperature 85 °C; plate equilibrium 0.05 min; sample equilibrium 10 min; mixing for 10 min at a mixing power of 5; stabilisation for 60 min.

The parameters of sample injection were: pressure generation for about 3 min and pressure equilibration within the vial for 0.1 min. The time to fill the sampling loop was set at 0.3 min and equilibration followed for 0.1 min. Duration of injection was about 2.5 min. The sampling loop was set on a temperature of 200 °C and transferline on 190 °C. The transfer line was splitless coupled with a 60 m capillary column (ID = 0.32 mm) named Rtx®-VGC (Fused Silica; Restek, Bad Homburg, Germany). The detection was performed using SCAN-mode within a mass range of m/z = 42-200 or SIM-Mode as described explicitly in the following. Electron bombardment ionisation was performed at 70 eV, temperature of the ion source was set at 230 °C and temperature of the quadrupole at 150 °C. As carrier gas helium 5.0 (Linde, Höllriegelkreuth, Germany) with a primary pressure of 83 kPa was used. The chosen temperature programme started at 40 °C for 2 min, followed by a heating rate of 5 °C/min to 155 °C and a second heating rate of 4 °C/min to 250 °C remaining at 250°C for 15 min. Data evaluation was done using HP ChemStation G1701 AA, Version A.03.00 with mass spectra library NBS 75K (data set entries: 75,000). An identification of a compound was only judged true, if the retention time as well as the ratio of intensities of quantifier and qualifier was conform. Quantification was generally done in SIM-mode.

12.0 g K_2CO_3 and 0.02 g NaN_3 were weighted in a 22 mL headspace-vial (IVA-Analysentechnik, Düsseldorf, Germany). At the field site 10 mL of groundwater were added and vial was directly closed with head-space caps with PTFE/Butyl septum and integrated snap ring (Perkin Elmer, Waltham, Massachusetts, USA). The closed vials were strongly

shaken to solve the main part of K_2CO_3. Until the analytics the samples were stored at -20 °C in the dark. To avoid losses, samples should be analysed within 2 weeks.

For the internal standard solution a mixture of deuterated compounds (toluene-D_8, naphthalene-D_8, furan-D_4, pyridine-D_5 and 2,5-thiophene-D_2) with a final concentration of 100 mg/L in methanol was prepared.

Before the analysis a volume of 10 µL of the mixture of a deuterated internal standard solution (100 mg/L) was added into the water phase of the sample, while the sampling vial was cooled in an ice bath. Vials were closed immediately, shaken and ultrasonicated to reach maximum in dissolved K_2CO_3. The amount of internal standard has to be adapted depending on the range of concentration of groundwater sample.

Stock solutions of compounds with a concentration of 20 g/L in methanol were prepared. Solutions were stored in headspace-vials at + 4°C in the dark. A mixture of all of the 32 investigated compounds was prepared in a concentration of 500 mg/L in methanol. Different dilutions were prepared in methanol.

For external standard solution 10 mL of non-contaminated groundwater (depth 2.5 m, Aachen-Hahn, Germany) were added to an initial weight of 12 g K_2CO_3 and 0.02 g NaN_3. Non-contaminated groundwater was used to simulate the field conditions as identical as possible. The calibration standards were prepared adding 10 µL of the diluted mixtures of stock solution in methanol. Furthermore, 10 µL of internal stocksolution were added. At the end, the volume of 20 µL of methanol should not be exceeded and should be the same in all vials. The procedure ends with shaking the closed headspace-vials and ultrasonication to solve high amounts of salts added.

3.3.6 Analyses of electron acceptors by ion chromatography

The analyses of nitrate, nitrite and sulfate was done by ion chromatography according to DIN 38405-19**.** The used instrument was a Dionex DX-100 (Dionex, Idstein, Germany). As precolumn Ion Pac AG 14 (4 mm × 50 mm, Dionex) and column Ion Pac AS 14 (4 mm × 250 mm, Dionex) were used. The autosampler (Spark Holland; Basic Marathon) with a loop volume of 50 µL had a preflush volume of 300 µL. The flow was about 1 mL/min, the analysis time about 14.5 min. A conductivity detector (thermal stabilizer, Dionex Model TS-2) with suppressor (Dionex, ASRS-ULTRA II, 4 mm) was used for the detection. Samples were filtrated (0.45 µm) and diluted before the analyses to reach concentrations within the calibrated range (nitrate and sulfate: 1-10 mg/L; nitrite: 0.1-1 mg/L).

3.3.7 Analyses of iron by ICP-MS

Dissolved iron was analysed by ICP-MS (Perkin Elmer SCIEX, ICP Mass Spectrometer, ELAN DRC II, Axial Field Technology) with AS 93 plus, Perkin Elmer as autosampler. Samples were filtrated using 0.2 µm filter (Spartan 13/0.2 RC, Whatman®, Schleicher & Schuell, Dassel, Germany). To avoid losses due to oxidation of Fe(II) and precipitation of $Fe(OH)_3$ samples were prepared immediately after sampling from anaerobic microcosms. 100 µL of the sample and 20 µl HNO_3 (Suprapur Merck, Darmstadt, Germany) were mixed. As internal standard rhodium standard solution (10 mg/L) (Merck, Darmstadt, Germany) was used. As standard the Certi PUR "ICP-Mehrelementstandardlösung VI" (Merck, Darmstadt, Germany) was used.

3.3.8 Photometric analyses of sulfide

The detection of sulfide was performed using the methylene blue method according to Cliné et al. (1969). 10 µL NaOH (10 M) were added to the filtrated samples (1 or 3 mL) followed by the addition of 1 mL Zn-acetate (200 g/L). 0.1 mL N,N-diethyl-1,4-phenylene-diammoniumsulfate (0.2 g/L in H_2O/H_2SO_4 (conc.) (50/50, v/v)) and 0.1 mL ammonia iron(III)sulfate × 12 H_2O (180 g/L) were added, mixed and filled with water to a final volume of 10 mL. After 10 minutes of incubation, samples were measured at an extinction wavelength of 670 nm. An 8 points containing calibration curve in the range of 0.21-10.50 µg S^{2-}/10 mL was performed.

3.4 Anaerobic degradation experiments

3.4.1 Anaerobic microcosms with groundwater of the Düsseldorf-Flingern site

Groundwater of well 19201 (7 m) taken in October 2004 was investigated for its microbial activity. Anaerobic conditions were indicated by low concentrations of oxygen (0.31 mg/L) and redox potential of -257 mV.

Using this groundwater, different microcosms were performed to reach sulfate-, iron-reducing as well as denitrifying conditions. In 1 L-bottles (Zschweile und Klinger, Hamburg, Germany) 1 mL SL10 solution (Widdel et al., 1983), 1 mL vitamin solution (Widdel and Pfennig, 1981), 1 mL NH_4Cl (2.5 g/L) and 1 mL Na_2HPO_4 (5 g/L) was filled. For every described composition of added agents, three abiotic controls were performed by the addition of 2 g/L NaN_3.

To achieve sulfate-reducing conditions $NaSO_4$ was added resulting in a concentration of 5 mM. Two different reducing agents were tested: titanium nitrilotriacetic acid (TiNTA) solution (prepared according to Moench and Zeikus, 1983) was added (1 mM final concentration) to three samples, while the other three samples were reduced by the addition of Na-dithionite (0.1 mM final concentration).

For iron-reducing conditions freshly precipitated iron hydroxide was added (2 g/L) (Beller et al., 1992). Six samples were reduced by the addition of 0.1 g/L ascorbic acid. Furthermore, to three of these samples 1 mL of anthraquinone-disulfonic acid was added (20 µM final concentration). In six other samples ascorbic acid was absent. Three of these contained 1 mL of anthraquinone-disulfonic acid.

For nitrate-reducing microcosms 10 mL of $NaNO_3$ (1 M) was added. Three samples were reduced by ascorbic acid (0.1 g/L), while the other three parallel samples were performed without ascorbic acid.

At the field site, 1 L of groundwater was added to the agents in the bottles. Bottles were closed immediately with Viton-rubber stoppers (Reichelt Chemietechnik GmbH+Co, Heidelberg, Germany) to avoid sorptive effects as well as butyl rubber seals (3 mm/35 mm; Macherey-Nagel GmbH & Co. KG, Düren, Germany). The air in the bottles was replaced by argon using a Rotanda syringe to flush the gas volume. Ti-NTA for sulfate-reducing microcosms was added by canula after bottles were flushed by argon. The blue-to violet complex was decolorized and served additionally as an oxidation-reduction indicator.

Samples were stored at room temperature over a time period of 2.5 years in a glove box (Toepffer Lab Systems, Göppingen, Germany) flushed with forming gas (N_2 95 %/H_2 5 %; Linde, Höllriegelkreuth, Germany). The glove box was covered with black curtain to avoid photooxic effects. 200 mL samples were taken at several times within 2.5 years of incubation. After sampling, the bottles were flushed with argon to replace the H_2 from the atmosphere in

the glove box. Furthermore, samples for the analyses of electron acceptors (nitrate, sulfate and iron) as well as sulfide were taken.

3.4.2 Anaerobic microcosms with groundwater of the Wülknitz site

Groundwater for sulfate- and iron-reducing microcosms originated from well IW1 (2-16 m), IW2 (depth not available), 1/97 (2-6 m), IW5 (4-16 m) and 13/01 (13-15 m) in Wülknitz. Samples were taken in July 2003, stored at 11 °C in the dark.

Microcosm studies were performed in July 2004 by S. Schönekerl and H. Lorbeer (University of Dresden). 1 L-glass bottles were filled completely without any headspace with groundwater. No nutrients were supplemented, while 0.1 g/L ascorbic acid was added as reducing agent. Because of the presence of high amounts of sulfate in the groundwater (80 mg/L in average), no additional sulfate was added. For achieving iron-reducing conditions 5 g amorphic iron hydroxide (Beller et al., 1992) were added per litre. For every groundwater sample and redox condition abiotic control microcosms were performed by the addition of 2 g/L NaN_3.

During the experimental time concentrations of nitrate and sulfate were measured by ion chromatography. Iron(II) was analysed using the photometric method according to DIN 38406-E1-1 (1983).

The bottles were stored in a Glovebox (Coy Laboratory Products Inc., USA), flushed with N_2 95 %/H_2 5 %, where also samples were taken after an incubation time of 1 and 2 years. Samples were acidified to pH ~2 and closed with teflon-coated caps and sent to Aachen, where sample-enrichment and measurements were performed. Limits of detection were about a factor 10 higher than described above, because only 100 mL of water sample were enriched.

3.4.3 Anaerobic degradation in on-site columns of the Wülknitz site

Four columns with a length of 150 cm and diameter of 20 cm were filled with aquifer material from well 17/03 (MP: 9-13 m and UP: 16-20 m depth). The aquifer material was homogenized by a concrete mixer, filled to the columns and loaded with groundwater of 17/03 MP since May 2004 to reach equilibrium conditions. Sampling was done by S. Schönekerl and H. Lorbeer (University of Dresden), while sample preparation and measurements were done in Aachen.

The four columns were positioned in a container tempered at 12 °C (Figure 9). Two columns were filled with groundwater from 17/03 MP, the other two ones with groundwater from 17/03 UP. One column of each was active to get information about microbial degradation, the other one was poisoned with 1.2 g/L sodium azide and used as abiotic control.

Sampling was done as follows: the groundwater was pumped regularly using MP1-immersion-pumps from well 17/03 MP as well as UP. Cloudy water was cleaned using activated carbon, and following piped to the canalisation. At the same time the first cycle of water leads to the pressure generation, and therefore the groundwater was not exposed to expansion before filled into the columns. Following, the four columns were filled with 3 L of groundwater of the respective depth (UP or MP). At the end of the columns 3 L of the groundwater were taken from every column for the analyses. After this procedure the columns were closed and exposed to pressure existing in the natural aquifer (1.5 bar UP; 0.6 bar MP).

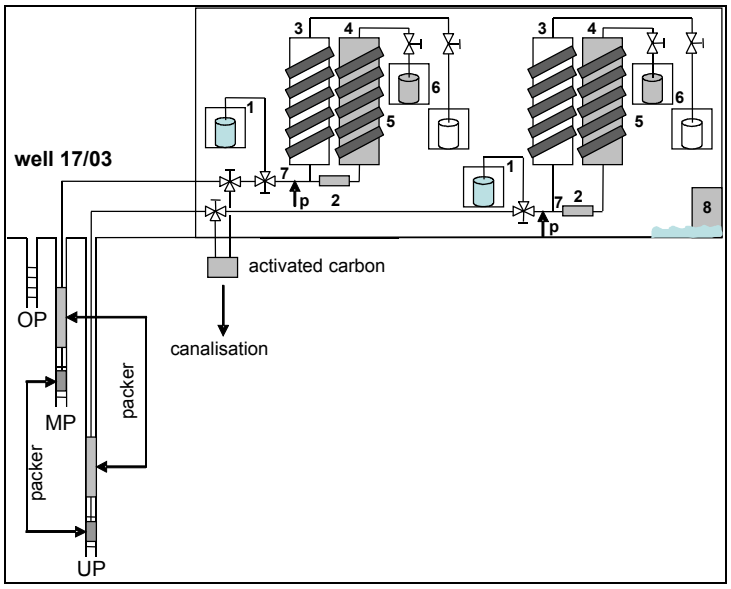


Figure 9: On-site column system at the Wülknitz site: 1) sampling unit before the columns; 2) poisoning unit; 3) active columns; 4) abiotic columns; 5) cooling system; 6) sampling unit after the columns; 7) pressure maintenance after sampling; 8) data logger (according to Hüsers et al., 2004).

Samples were taken 10 times in the period of April 2005 to February 2006. The first 6 sampling campaigns were done in an interval of 4 weeks, the last 4 in an interval of 6 weeks. The kinematical porosity of the aquifer material is about 0.19. Therefore, the groundwater remained in the columns about 84 days (4 week sampling interval) and 126 days (6 week sampling interval).

3.4.4 Anaerobic microcosms to determine the degradability of quinoline compounds

All microcosms were performed in 100 mL serum bottles (Wheaton, Millville NJ, USA) containing 55 mL of medium and 5 g of aquifer material. The microcosm bottles were flushed with N_2/CO_2 (80/20; v/v) and closed with Viton rubber stoppers (Maag Technik, Dübendorf, Switzerland) to avoid sorption. The flasks were incubated at room temperature in the dark.

Commercially available chemicals of highest purity were used (see chapter 7.1, appendix). Stock solutions of quinoline compounds in water were degassed and flushed repeatedly with nitrogen to eliminate dissolved oxygen. Stock solutions (1-4 mL) were added to the medium by a syringe, resulting in a final concentration of approx. 80 µM. For abiotic controls the soil was autoclaved twice and supplemented with sodium azide (2 g/L).

A mineral bicarbonate-buffered freshwater medium, containing NaCl (1.0 g/L), $MgCl_2 \times 6\ H_2O$ (0.4 g/L), KH_2PO_4 (0.2 g/L), NH_4Cl (0.25 g/L), KCl (0.5 g/L) and $CaCl_2 \times 2\ H_2O$ (0.15 g/L) was prepared under N_2/CO_2 (80/20; v/v) atmosphere (Biogon C20, Linde, Höllriegelskreuth, Germany). $NaHCO_3$ (2.52 g in 30 mL H_2O) was autoclaved under saturation of CO_2 and added to the mineral medium (Widdel and Bak, 1992). This medium was supplemented with 1 mL of 7-vitamin solution (Widdel and Pfennig, 1981), 1 mL of trace element solution (SL 10) and 1 mL of selenite-tungstate solution per litre (Widdel et al., 1983). The pH was adjusted to 7.2-7.3 using an anoxic Na_2CO_3 solution (0.5 M).

The sterile filtrated solution of 7-vitamin-solution consisted of vitamin B_{12} (50 mg/L), 4-aminobenzoic acid (50 mg/L), D(+)-biotin (10 mg/L), nicotinic acid (100 mg/L), Ca-D(+)-panthothenate (25 mg/L), pyridoxamine dihydrochloride (250 mg/L), thiamine dihydrochloride (50 mg/L). The solution was stored at 4 °C in the dark. Autoclaved trace

element solution was composed of $FeCl_2 \times 4\ H_2O$ (1500 mg/L), $ZnCl_2$ (70 mg/L), $MnCl_2 \times 4\ H_2O$ (100 mg/L), $CoCl_2 \times 6\ H_2O$ (190 mg/L), $CuCl_2 \times 2\ H_2O$ (2 mg/L), $NiCl_2 \times 6\ H_2O$ (24 mg/L), $Na_2MoO_4 \times 2\ H_2O$ (36 mg/L), H_3BO_3 (6 mg/L), HCl 25 % (10 mL/L). Selenite-tungstate solution was composed by NaOH (500 mg/L), $Na_2SeO_3 \times 5\ H_2O$ (3 mg/L) and $Na_2WO_4 \times 2\ H_2O$ (4 mg/L) and autoclaved before usage.

To obtain sulfate-reducing conditions, Na_2SO_4 (5 mM) was added as electron acceptor to the medium above, as well as ferrous sulfide to eliminate traces of oxygen by addition of sodium sulfide solution (1 mM final concentration) and Fe(II) chloride solution (3 mM final concentration). Fe(II) was present in excess to avoid toxic effects of sulfide.

To obtain iron-reducing conditions, iron hydroxide (2 g/L) was added from a suspended anoxic stock solution. It was prepared from $FeCl_3$ (6.5 g) dissolved in 100 mL of water, which was adjusted to pH 7 by NaOH. The iron hydroxide formed was washed and resuspended in water (Beller et al., 1992). The assays were further supplemented with sulfate (0.5 mM) as sulfur source and anthraquinone disulfonic acid (20 µM) as electron shuttle (Lovley et al., 1996).

The nitrate-reducing medium contained sulfate (0.5 mM) as sulfur source and nitrate (5 mM) as electron acceptor.

The aquifer material for microcosms originated from the former coke manufacturing site in Castrop-Rauxel, Germany (well T3, depth ~3.5 m; Figure 5). After sampling, the water-saturated aquifer material was stored at 4 °C in the dark until the experiments. Extraction of the used sample of aquifer material with acetone or water by ultrasonication showed no detectable concentrations of tar oil compounds (LOD: 0.2 µg/kg).

Sampling in microcosms was conducted at least over a period of 300 days, using plastic syringes flushed with N_2/CO_2 (80/20; v/v); samples were filtrated through a 0.2-µm filter (Spartan 13/0.2 RC, Whatman®, Schleicher & Schuell, Dassel, Germany) into HPLC vials. No adsorption of the parent compound and identified metabolites to the syringe or filter material was observed. The filtrated samples were analysed directly or stored at 4 °C until the analysis.

3.5 Toxicity tests

3.5.1 Ecotoxicity tests

As an ecotoxicicity test the luminescence-inhibition test with *Vibrio fischeri* was conducted twice for each concentration by using the standard procedure according to ISO 11348-1 (1998). The growth-inhibition test with the algae *Desmodesmus subspicatus* and the *Daphnia magna* Straus immobilization test were conducted following standard procedures (ISO 8692, 2004 and ISO 6341, 1996) in triplicate and fourfold per concentration tested, respectively. The algae growth inhibition test was carried out in 24 well microplates (Eisentraeger et al., 2004) with a final test volume of 200 µL/well. Growth inhibition assays with *Vibrio fischeri* and *Pseudomonas putida* were performed using computer controlled microplate incubators and photometers (IEMS-reader™, Thermo Life Sciences, Basingstoke, UK). The growth inhibition test with *Vibrio fischeri* was based on DIN 38412 L37 (1999) and the test with *Pseudomonas putida* on ISO 10712 (1995).

The EC_{50}-values were calculated from the sigmoid concentration-inhibition curves fitted by probit analysis using the maximum likelihood solution (Finney, 1964). Confidence intervals were calculated by the equation from Finney (1964) and Weber (1986).

3.5.1.1 Stock solutions and quantification within the toxicity tests

All chemicals used were of purity higher than 97 % (see chapter 7.1, appendix). 500 mL of each stock solution in Millipore®-water (Millipore, Schwalbach, Germany) was prepared and stored in Duran® glass bottles (Schott Rohrglas, Mitterteich, Germany) at 4 °C for up to one month in the dark.

In the algae growth inhibition test and the daphnids immobilization as well *Pseudomonas putida* and *Vibrio fischeri* growth inhibition test three concentrations of compound tested at the beginning and at the end of the test were analysed using HPLC-DAD-measurements to determine the real concentrations in the test treatments according to OECD 201 (2006). Furthermore, three test-concentrations were prepared without the addition of media and test organisms. The nominal concentrations used in the tests were corrected using a plot of the nominal concentrations versus the average of concentrations (beginning and final concentration) of the test. These corrected concentrations were used for the probit analysis to assess effective concentration values (EC_{50}-values).

3.5.2 Genotoxicity tests

3.5.2.1 *UmuC* test

Genotoxicity was determined using the *umuC* test with *Salmonella enterica* serovar Typhimurium TA1535/pSK1002 which is based on the induction of the SOS response as a direct relation to the degree of DNA damage (Oda et al., 1985, 1995). The test was performed in microplates according to ISO 13829 (2000) performing three parallels and test treatments with and without metabolic activation (S9 microsome fraction, S9-mix). The S9 microsome fraction is induced with Aroclor 1254 (Trinova Biochem, Gießen, Germany) and is able to simulate eukaryotic metabolism processes that cannot be conducted by the microorganisms themselves.

A test sample or a test dilution was evaluated as genotoxic if the induction rate exceeds the value of 1.5 and the growth factor is equal to or greater than 0.5 (ISO 13829, 2000).

3.5.2.2 Ames fluctuation test

The Ames fluctuation test was conduced according to the method of Reifferscheid et al. (2005) using *Salmonella enterica* serovar Typhimurium TA98 and TA100. Bacterial strains were grown overnight in Oxoid broth no. 2 shaking at 37 °C. To select for plasmid pKM101 ampicillin (50 mg/L) was added. When reaching the appropriate optical density the cultures were exposed to the test material. The dilutions of the test samples were pre-incubated in triplicate with and without S9-mix for 100 min at 37 °C under shaking. Thereafter the mixtures were diluted six-fold with histidine-deficient minimal medium, containing bromocresol purple as a pH-indicator followed by a subsequent selection phase at 37 °C for 48 h.

A colour change of the pH indicator as a consequence of bacterial growth indicates reversions. Substances were considered to be mutagenic, if they produced statistically significant concentration-dependent increases in the number of reverting wells. The results were evaluated by nonparametric χ^2-test ($P < 0.05$) as recommended by Green et al. (1976) and Gatehouse (1978).

3.6 Sorption of quinoline compounds to aquifer material and montmorillonite

Batch experiments with aquifer material from the Castrop-Rauxel site were conduced with non-contaminated material sieved to 2 mm (coarse sand: 0.63 %, medium sand: 72.83 %, fine grained sand: 22.35 %, coarse silt : 2.63 %, medium silt: 0.83 %, fine silt: 0.50 %, clay: 0.25 %; carbon content: < 0.08 mg/g) in 20 mL-vials. Aquifer material was sterilized by applying a gamma radiation dose of 60-70 kGay (Fa. Studer Kabel, Aarau, Switzerland).

As clay mineral Ca-montmorillonite (type Cheto, Saz-1; Apache County, Arizona USA; CAS: 1302-78-9, Clay Mineral Society, Colorado, USA) was used. The composition is described by $(Ca_{0.36}Na_{0.36}K_{0.02})$ $[Al_{2.71}Mg_{1.11}Fe(III)_{0.12}Mn_{0.01}Ti_{0.03}]$ $[Si_{8.00}]O_{20}(OH)_4$. It is characterized by a cation capacity of 1.2 mol/kg and a BET-surface of $97.42 \pm 0.58 m^2/g$. The preparation of montmorillonite was done by washing twice with 0.5 M KCl and twice with 1 M KCl. The washed montmorillonite was used for the experiments after 24 h.

For all experiments with aquifer material and montmorillonite, synthetic groundwater with a molarity of ~0.01 M Ca^{2+} was used. The composition was as follows: $CaSO_4 \times 2\ H_2O$: 1.39 g/L; $CaCl_2$: 0.05 g/L; NH_4Cl: 0.03 g/L; $MgSO_4 \times 7\ H_2O$: 0.56 g/L.

The following ratios of montmorillonite and water phase were used in all of the experiments: quinoline and 6-quinolinol: 1:300; 2(1H)-quinolinone, 1(2H)-isoquinolinone, 3,4-dihydro-2(1H)-quinolinone, 4(1H)-quinolinone: 1:5. The ratio of aquifer material and water phase was 1:1 for all compounds investigated. Ratios of 1:300 were performed in 100 mL brown-glass bottles (Schott-Duran, VWR International, Darmstadt, Germany) closed with Teflon-coated caps (Supelco, Munich, Germany), ratios of 1:5 in brown glass bottles of a volume of 20 mL.

In general the batches were shaken for 24 h (for kinetics several days). Batches were done in triplicate. pH-values were controlled at the beginning as well as at the end of the experiments and were adjusted if necessary by the addition of minor amounts of 20 M NaOH or HCl. Filtered water phase was analysed with HPLC-UV at 210 nm.

A concentration of 1 mg/L was investigated in batches to determine kinetics, the influence of pH, ionic strength as well as cationic composition.

The influence of pH on sorption was investigated by setting the solution to 5 different pH-values in the range of pH 3-10. For testing the influence of cationic composition montmorillonite was washed with 0.5 and 1 M solutions of $CaCl_2$ as described above. In batches studying the influence of ionic strength, the amount of $CaCl_2$ in synthetic groundwater was increased to 10.21 g/L, resulting in a solution of ~ 0.1 M Ca^{2+}.

For isotherms a range of concentration of 0.1–100 mg/L (0.1, 0.3, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 20, 30, 40, 50, 60, 80, 100 mg/L) was investigated. Each concentration was tested in triplicate.

3.7 Extraction of aquifer material

For the extraction of aquifer material, 10 mL of organic solvent was added to 1 g aquifer material from a well in the higher contaminated region of the testfield (T12) in Castrop-Rauxel (Figure 5), spiked with D_7-quinoline, and extracted using ultrasonication for 1 h. Different organic solvents (acetonitrile, methanol, acetone, acetone/methanol (50/50; v/v), ethyl acetate, tetrahydrofurane or DMSO) were used to check the effect of extraction. The extracts were filtrated through a 0.2 µm filter (Spartan 13/0.2 RC, Whatman®, Schleicher & Schuell, Dassel, Germany) into HPLC-vials. The analyses of different extracts showed that extraction was not significantly discriminated by the choice of organic solvent. As consequence data of all seven extracts were averaged.

3.8 Analyses of tar oil constituents

Commercially available tar products were analysed for the presence of hydroxylated quinoline compounds. Tar oil no. 1 (type WEI B), no. 2 (type WEI C), and no. 3 (type WEI C) originated from an active wood-impregnation site. Tar oil no. 4 (type WEI B) and coal tar no. 5 were a gift of Rüttgerswerke (Castrop-Rauxel, Germany). Tar oil no. 6 originated from a former impregnation site and was skimmed from groundwater and soil. Coal tar 7 was purchased from Alfa Aesar (Karlsruhe, Germany). Coal tars and tar oils were diluted in acetone/methanol (50/50; v/v) and treated by ultrasonication. The dissolved portion was separated and used for analysis. Average data of three dilutions were formed.

3.9 Determination of water solubility

Saturated solutions of quinoline compounds in water were ultrasonicated and shaken for 3 h. Suspensions were stored over night at room temperature (20 °C). Saturated solutions were centrifuged for 15 min with 5000 RPM (Labofuge^{GL}, Heraeus, Kendo, Hanau, Germany). Supernatants were filtrated through 0.2 µm filters (Spartan 13/0.2 RC, Whatman®, Schleicher & Schuell, Dassel, Germany) after tempering to 20 °C into HPLC-vials. Every compound was investigated fivefold. Dilutions prior the analysis were done with water (1:500, 1:1000 or 1:2000). HPLC-measurements on an HP1100 (Agilent®, Waldbronn, Germany) with UV-diode array (detection wavelength λ = 210 nm) were performed using external calibration for the quantification (14 standard solutions in water, 0.2-30 mg/L). Coefficients of linear regression were always R^2=0.999.

3.10 Determination of tar oil/water partitioning

Different ratios of tar oil and water have been investigated to avoid dependence of partition coefficients on solute concentration. The experiments were carried out in 20 mL glass vials, with 0.5 g tar oil and 10 mL $CaCl_2$-solution (0.01 N). The vials were closed with Teflon-coated caps, ultrasonicated for 1 h, followed by shaking for 12 h at room temperature. The two phases were separated by centrifugation. An aliquot of the water phase was carefully removed to avoid carry-over of the organic phase. After removing whole water-phase an aliquot of the remaining tar oil was taken and diluted in acetone/methanol (50/50; v/v) for the analyses by HPLC-MS-MS.

4. RESULTS AND DISCUSSION

4.1 Analytics of heterocyclic compounds

4.1.1 Analytics of quinoline compounds using HPLC-MS-MS

N-heterocycles like quinoline compounds are well known as contaminants in tar oil contaminated groundwater at former gasworks sites, coke manufacturing sites as well as wood impregnation sites. To estimate the degree of contamination as well as potential environmental risk of contamination at these sites a reliable analytical method was needed to determine both the contaminants and the possible polar metabolite. Therefore a selective and sensitive analytical method had to be performed.

Until now, published methods for the identification of quinoline compounds were mostly based on GC-MS or GC-FID measurements (Pereira et al., 1983 and 1987; Johansen et al., 1997b). With these methods non-substituted as well as non-polar, substituted quinolines could be analysed, while more polar quinoline compounds and known metabolites -hydroxylated quinoline compounds- were detectable only with low sensitivity. Therefore, Mundt and Hollender (2005) performed a robust HPLC-DAD method to determine a huge number of heterocyclic compounds as well as their metabolites. However, the usage of a DAD-detector for the detection and identification of compounds is limited: the interferences of the UV-detection due to coeluting substances, low selectivity and sensitivity of compounds are the drawback of the method.

Edler et al. (1997) used an HPLC-MS method for the non-target screening of tar oil contaminated groundwater. The presence of several polar N-heterocyclic compounds even as major contaminants was indicated. Therefore, the demand of a reliable HPLC-MS-MS method for the determination of a huge number of different substituted quinoline compounds as well as their metabolites in groundwater was evident.

4.1.1.1 Validation of the HPLC-MS-MS method

The instrumental HPLC-MS-MS system as well as separation conditions were used as described in chapter 3.3.2. A triple quadrupole mass spectrometer equipped with electrospray ionisation was applied for the detection. The ionisation was performed using positive mode of ionisation. For all quinoline compounds the protonated molecular ion $[M + H]^+$ was selected as precursor ion. Detection was performed in the multiple reaction monitoring mode using the most intense and specific fragment ion (and for most compounds a second fragment for further confirmation) together with the retention time for the identification of compounds. Concrete settings (retention time (r_t), precursor ion and fragments, limit of qualification (LOD) and limit of quantification (LOQ)) are given in Table 3. In Figure 10 the chromatographic separation as well as the single transitions of quinoline compounds of a standard solution (400 µg/L) are shown, gradients are given in chapter 3.3.2.

Table 3: MS-MS parameter of the quinoline method.

compound	r_t [min]	precursor ion	fragments			
			quantifier		qualifier	
			[m/z]	[V]	[m/z]	[V]
quinoline	22.5	130	77	DP: 55 CE: 45	103	DP: 60 CE: 35
isoquinoline	23.7	130	77	DP: 55 CE: 45	103	DP: 60 CE: 35
1-methylisoquinoline	25.5	144	103	DP: 55 CE: 35	-	-
3-methylisoquinoline	26.1	144	115	DP: 55 CE: 45	103	DP: 55 CE: 35
2-methylquinoline	24.5	144	103	DP: 55 CE: 35	115	DP: 55 CE: 45
4-methylquinoline	24.8	144	115	DP: 55 CE: 45	103	DP: 55 CE: 35
6-methylquinoline	25.7	144	115	DP: 55 CE: 45	-	-
2(1H)-quinolinone	13.6	146	128	DP: 50 CE: 30	77	DP: 60 CE: 30
1(2H)-isoquinolinone	14.5	146	77	DP: 50 CE: 45	-	-
4(1H)-quinolinone	5.8	146	77	DP: 50 CE: 45	118	DP: 50 CE: 45
5(1H)-quinolinone	12.4	146	118	DP: 50 CE: 30	-	-
6-quinolinol	10.8	146	118	DP: 50 CE: 30	128	DP: 60 CE: 30
7-isoquinolinol	15.2	146	118	DP: 50 CE: 30	-	-
3(2H)-isoquinolinone	9.6	146	128	DP: 60 CE: 30	77	DP: 60 CE: 30
3,4-dihydro-2(1H)-quinolinone	16.2	148	106	DP: 55 CE: 35	-	-
3,4-dihydro-1(2H)-isoquinolinone	12.8	148	105	DP: 36 CE: 25	-	-
2-methyl-6-quinolinol	14.0	160	117	DP: 55 CE: 35	115	DP: 60 CE: 30
4-methyl-2(1H)-quinolinone	16.7	160	115	DP: 60 CE: 30	117	DP: 55 CE: 35
6-methoxyquinoline	21.2	160	117	DP: 55 CE: 35	-	-
1-methyl-2(1H)-quinolinone	17.4	160	117	DP: 55 CE: 35	115	DP: 60 CE:30
2-methyl-4(1H)-quinolinone	7.5	160	117	DP: 55 CE: 35	115	DP: 55 CE: 35
2,4-dimethylquinoline	28.5	158	143	DP: 55 CE: 35	115	DP: 55 CE: 40
2,6-dimethylquinoline	29.2	158	115	DP: 55 CE: 40	143	DP: 55 CE: 35
1,2,3,4-tetrahydroquinoline	24.0	134	106	DP: 36 CE: 27	-	-
7-hydroxy-4-methyl-2(1H)-quinolinone	7.2	176	103	DP: 55 CE: 31	134	DP: 55 CE: 31
4-hydroxy-1-methyl-2(1H)-quinolinone	4.1	176	134	DP: 55 CE: 31	103	DP: 45 CE: 40
D_7-quinoline	22.5	137	109	DP: 31 CE: 37	-	-

r_t = retention time; DP = declustering potential; CE = collision energy; CXP = 10 V, exit potential of collision chamber; EP = 10 V, entrance potential; data determined with API3000 as instrument.

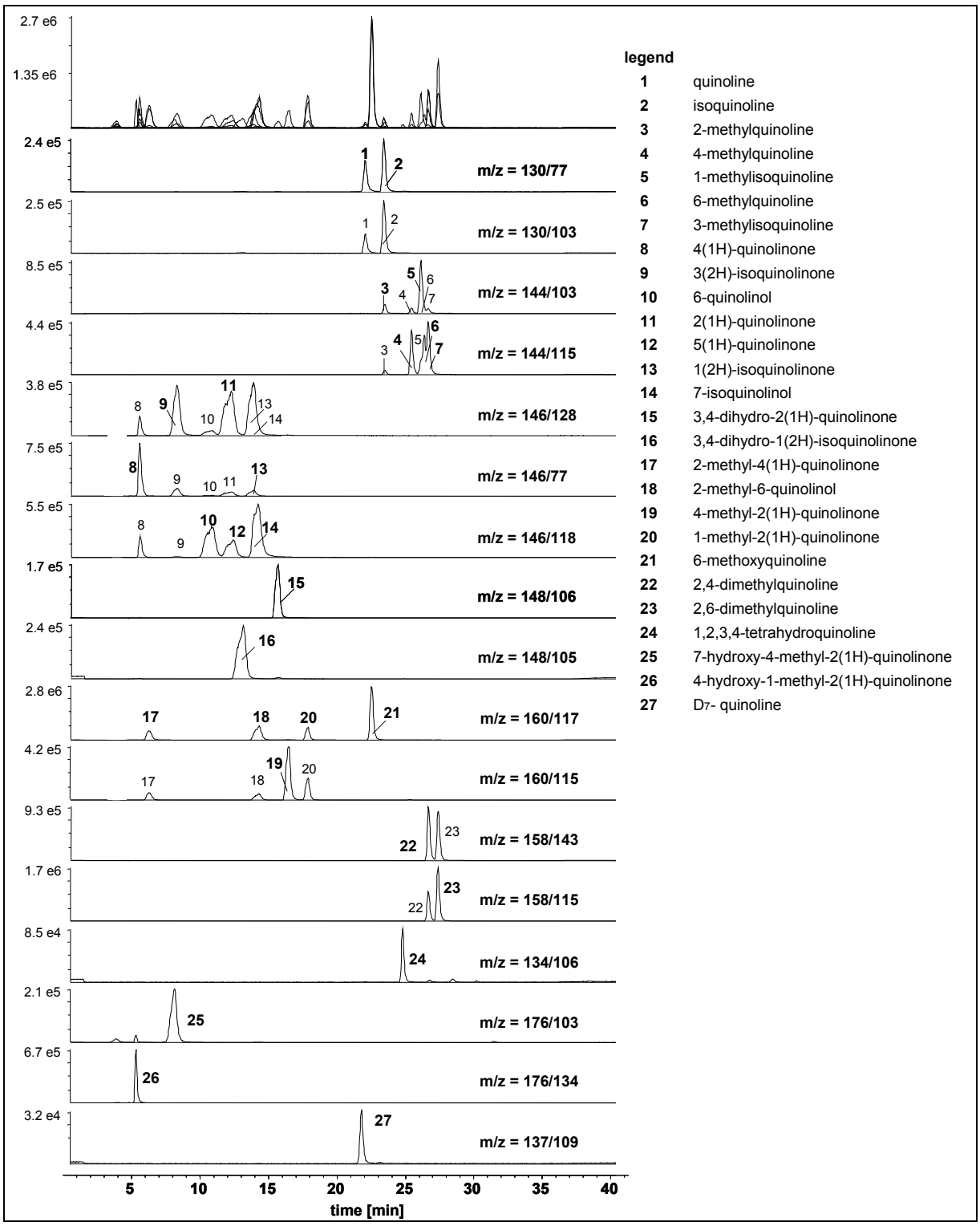


Figure 10: Chromatogram of a standard-mixture (400 µg/L) using the HPLC-MS-MS method. For chromatographic gradient see chapter 3.3.2. The transition used for the quantification is marked by a bold number.

The applicability of the method using SPE-enrichment with Isolute ENV$^+$-cartridges, described by Mundt and Hollender (2005), was tested for quinoline compounds.

For the determination of recoveries, limit of qualification and quantification non-contaminated groundwater from Aachen-Hahn, Germany was used. 1 L of the non-contaminated groundwater was spiked with different amounts of standard mixture (3 levels: 20, 200 and 1000 µg/L final concentration, 7 parallels each) and prepared as described in chapter 3.2 to determine recoveries. Furthermore, to get standards in matrix 13 groundwater samples were spiked with different amounts of quinoline mixture and prepared using the described SPE-procedure. Limits of qualification as well as limits of quantification were calculated on the basis of DIN 32645 (1994). Therefore, seven 1 L-samples (n = 3) were spiked (0.1, 0.5, 1, 2, 5, 7.5, 10 µg/L in methanol) and enriched using SPE-procedure.

Results of recoveries as well as LOD and LOQ are shown in Table 4. The procedure allows to determine the quinoline compounds in the lower ng/L-range. A comparison of the calibration curves of directly diluted standards and of standards prepared using SPE-enrichment were in good accordance showing the robustness of the system and the possibility to use standards without matrix for the quantification (Table 24, appendix). Linearity was found up to concentrations of 1000 µg/L.

Table 4: Recovery rate (R.R.), relative standard deviation (R.S.D), limit of detection (LOD) and limit of quantification (LOQ) of the analytical method.

compound	R.R. (%)	R.S.D (%)	R.R. (%)	R.S.D (%)	R.R. (%)	R.S.D (%)	LOD	LOQ
	20 µg/L		200 µg/L		1000 µg/L		[µg/L]	[µg/L]
quinoline	102	± 7	91	± 11	90	± 6	0.006	0.023
isoquinoline	134	± 10	89	± 12	90	± 6	0.014	0.049
1-methylisoquinoline	83	± 13	90	± 10	85	± 5	0.01	0.034
3-methylisoquinoline	110	± 6	91	± 12	88	± 7	0.004	0.027
2-methylquinoline	103	± 8	93	± 13	92	± 5	0.006	0.022
4-methylquinoline	101	± 11	91	± 13	90	± 5	0.004	0.013
6-methylquinoline	101	± 9	93	± 12	89	± 5	0.004	0.016
2(1H)-quinolinone	95	± 2	89	± 12	92	± 6	0.010	0.034
1(2H)-isoquinolinone	95	± 7	79	± 12	83	± 7	0.034	0.127
4(1H)-quinolinone	103	± 10	94	± 13	87	± 4	0.008	0.027
5(1H)-quinolinone	76	± 9	81	± 12	73	± 8	0.007	0.026
6-quinolinol	107	± 3	91	± 13	90	± 5	0.004	0.015
7-isoquinolinol	87	± 6	80	± 13	75	± 5	0.004	0.016
3(2H)-isoquinolinone	105	± 6	90	± 14	93	± 6	0.004	0.016
3,4-dihydro-2(1H)-quinolinone	98	± 5	88	± 9	89	± 6	0.008	0.027
3,4-dihydro-1(2H)-isoquinolinone	101	± 4	93	± 13	91	± 5	0.006	0.020
2-methyl-6-quinolinol	102	± 7	97	± 14	96	± 5	0.003	0.011
4-methyl-2(1H)-quinolinone	98	± 2	90	± 13	92	± 5	0.006	0.020
6-methoxyquinoline	101	± 4	92	± 12	90	± 6	0.003	0.009
1-methyl-2-quinolinone	104	± 4	91	± 12	92	± 5	0.005	0.018
2-methyl-4(1H)-quinolinone	117	± 4	99	± 12	89	± 6	0.006	0.023
2,4-dimethylquinoline	87	± 14	90	± 10	85	± 4	0.006	0.022
2,6-dimethylquinoline	95	± 13	92	± 12	83	± 5	0.006	0.020
1,2,3,4-tetrahydroquinoline	71	± 14	76	± 16	89	± 6	0.013	0.045
7-hydroxy-4-methyl-2(1H)-quinolinone	115	± 5	94	± 15	93	± 4	0.005	0.018
4-hydroxy-1-methyl-2(1H)-quinolinone	124	± 17	96	± 17	102	± 7	0.006	0.023
D_7-quinoline	101	± 4	89	± 13	91	± 5	0.003	0.019

4.1.1.2 Discussion of the HPLC-MS-MS method

Solid-phase extraction with Isolute ENV$^+$-cartridges according to Mundt and Hollender (2005) was successfully enlarged on a huge number of differently substituted quinoline compounds. Therefore, only one SPE-enrichment is necessary to determine the spectrum of NSO-heterocyclic and quinoline compounds.

HPLC coupled with tandem mass spectrometry allowed the sensitive and selective analyses of quinoline compounds in groundwater, even in minor amounts (LODs in the lower ng/L-range), resulting in the possibility to analyse groundwater samples of tar oil contaminated sites along the plume of contamination over a long distance. Also more polar compounds like hydroxylated quinolines, which are mentioned as metabolites are included into this method. Therefore, this method can be used to search for indications of microbial degradative natural attenuation processes.

D_7-quinoline is the only labelled quinoline compound which is commercially available until now. It could not be used as an internal standard for whole quinoline compounds, but its peak area was monitored over whole series of measurement to check the SPE-enrichment, to control the instruments performance during the measurement, and at least used as a marker of retention time. However, it is indisputable that there is a demand to produce labelled quinoline compounds to get more reliable data, especially if quinoline compounds will be included in legal demands of groundwater controlling.

The HPLC separation does not allow assigning the position of a methyl group for several methylsubstituted quinolines as well as methyl-2(1H)-quinolinones. Therefore, data obtained for these two groups of compounds, shown in the following chapters, have to be taken with care and are a result of comparison of retention times with external standards. Separation techniques reaching higher resolution and therefore identification of important isomers may be the subject of the future, if isomer composition is of interest.

Various substituted quinoline compounds were detected in the groundwater of three tar oil contaminated sites, which is shown in the following chapter 4.2.

4.1.2 Analytics of volatile NSO-heterocycles using Headspace-GC-MS

In addition to 2-, 3-ring and more ring aromatic compounds, monocyclic compounds are also known as tar oil constituents and were also found in tar oil contaminated groundwater (Lang and Eigen, 1967; Stuermer et al., 1982; Johansen et al., 1997b; Zwiener and Frimmel, 1998). However, the occurrence of monocyclic heteroaromatic compounds in tar oil contaminated groundwater was only scarcely studied until now. Routinely tar oil contaminated groundwater is analysed for the group of BTEX-compounds which are <1 % present in tar products (Meyer et al., 1999). Analyses of BTEX are often performed by Headspace-GC-FID or -MS using the volatile characteristic of these compounds (DIN 38407 F9-1, 1991). Therefore, an analytical method should be developed, which allows the identification and quantification of monoaromatic heterocyclic compounds in addition to BTEX and volatile homocyclic compounds.

4.1.2.1 Headspace-GC-MS method validation

The instrumental as well as chromatographic parameters are given in chapter 3.3.5, while here the most important details are mentioned.

The used temperature program allowed the separation of a broad number of different compounds as shown in Figure 11. The assignment of compounds is given in Table 5. Although the chromatographic separation was achieved for most compounds without any problem, it was not possible for the following couples: *m*-xylene/*p*-xylene/2,5-dimethyl-thiophene; 2-methylnaphthalene/2-methylbenzothiophene; 1-methyl-naphthalene/3-methyl-benzothiophene as well as 4-methylquinoline/1,3-dimethyl-naphthalene.

It was found of high necessity to set the temperature of the transfer line to 190 °C to avoid memory effects of the less volatile compounds (e.g. acenaphthene, dibenzofuran, indole, quinoline) as they were found at a temperature of 110 °C. Furthermore, this change also let to a better reproducibility of the instruments sensitivity (13$\pm$8 % in contrast to 20$\pm$8 %; n = 10).

K_2CO_3, out of a high number of salts tested (NaCl, K_2CO_3, $CaSO_4$, $Al_2(SO_4)_3 \times H_2O$, $MgCl_2 \times 6\ H_2O$, $MgSO_4 \times 7\ H_2O$, Na_2SO_4), was found as the one leading to highest sensitivity when added in amounts resulting in a saturated solution. Furthermore, the addition of K_2CO_3 led to the positive effect of basic pH in the solution (pH ~13). This effect was found to be positive especially for N-heterocyclic compounds, resulting in an increased sensitivity.

The usage of the SIM-mode instead of the scan-mode (m/z = 42-200; 1.21 scans/s) yielded in an increase of sensitivity for all compounds with a factor of 32.

Limits of qualification and quantification were performed according to DIN 32645 (1994). 12 dilutions in the range of 0.1-25 µg/L were prepared. The results are shown in Table 5. LODs were found within the range of 0.5-6.6 µg/L. Highest limits of detection were found for high molecular weight compounds like the 3-ring aromatic compounds acenaphthene, dibenzofuran as well as for N-heterocycles such as indole, quinoline as well as their dimethylated analogs.

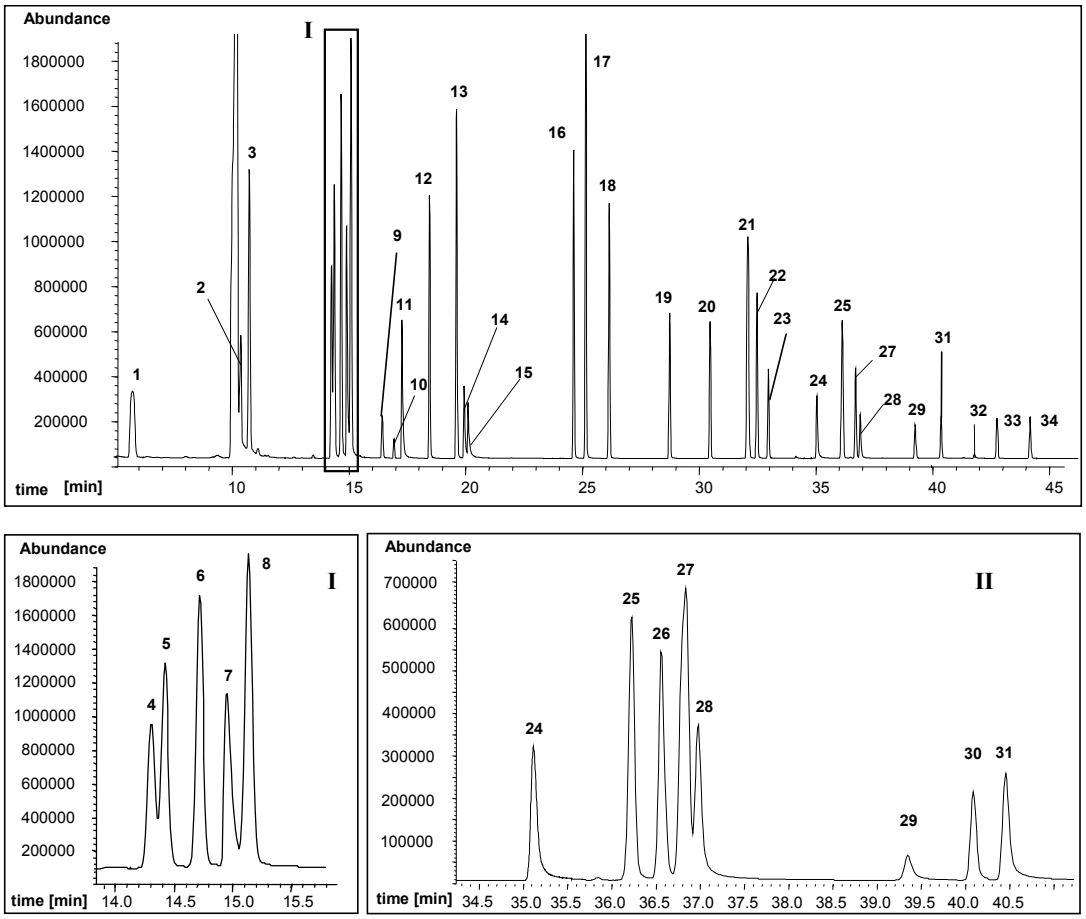


Figure 11: Chromatogram of the standard mixture (100 µg/L) in the SIM-mode. I: Cut-out of the time range 14.0-15.5 min. II: Cut-out (34.0-41.0 min) of a chromatogram of the standard mixture of methylbenzothiophenes. For peak numbering see Table 5.

For the evaluation of the linear range of detection standard solutions were diluted within 1-100 µg/L (5 dilutions, 3 parallels). The plots of detected peak area and concentration are shown in Figure 47. For all compounds investigated high linearity was found in the range of 1-100 µg/L, shown by the linear correlation coefficients (R^2), mostly higher than 0.98.

To find the best method of quantification, external calibration, internal calibration with deuterated compounds, the standard addition and the MHE method (5 extractions) were compared. Therefore, solutions in the concentration range of 1-100 µg/L were prepared using non-contaminated groundwater (Aachen-Hahn, Germany). For external calibration concentrations of 1, 25, 50, 75, 100 µg/L were measured. Peak areas were directly correlated to the respective concentration yielding a calibration curve, which was used to evaluate a standard solution of 50 µg/L. For the internal calibration (external calibration corrected with internal standards), the same standard dilutions were used, but data evaluation was done using internal standards. 100 µg/L internal standard mixture was added to each dilution. For the standard addition method 10 µL of stock solution with different concentrations (25, 50, 75 and 100 µg/L) was added to a starting concentration of 25 µg/L. Evaluation was done in triplicate without using internal standards. The MHE method included 5 times an injection from the same vial. The decrease in concentration (50 µg/L) with number of injection was correlated to another standard solution (25 µg/L) using MHE-calculation program (Perkin Elmer, 1993). Measurements were done in triplicate. To assess the results, linear correlation coefficients (R^2) were calculated. The variances (%) from nominal concentration was used as

a parameter assessing the quality of the quantification method. This approach was done for selected compounds exemplarily. Data are shown in Table 6.

Table 5: Parameters of the HS-GC method.

	compound	r_t [min]	quantifier [m/z]	qualifier [m/z]	LOD [µg/L]	LOQ [µg/L]	time range [min]
1	furan	5.9	68	39	1.4	6.9	5.0 – 10.0
	furan-D_5	5.9	72	44	1.4	6.9	
2	benzene	10.4	78	51	0.8	3.6	10.0 – 14.0
3	2,5-thiophene-D_2	10.8	86	59	-	-	
	thiophene	10.8	84	58	-	-	
4	toluene-D_8	14.3	98	70	0.4	2.1	14.0 – 16.2
5	toluene	14.4	91	65	0.4	2.1	
6	2-methylthiophene	14.7	97	45	0.8	3.6	
7	pyridine	15.0	79	52	2.7	13.1	
	pyridine-D_5	15.0	84	56	2.7	13.1	
8	3-methylthiophene	15.1	97	45	0.7	3.4	
9	tetrahydrothiophene	16.9	88	60	0.9	4.4	16.2 – 18.2
10	pyrrol	17.0	67	39	-	-	
11	2-methylpyridine	17.3	93	66	-	-	
12	2,5-dimethylthiophene	18.5	111	97	0.6	2.9	18.2 – 19.2
13	*o*-xylene	19.7	91	106	0.7	3.2	19.2 – 23.4
14	3-methylpyridine	20.0	93	66	-	-	
15	4-methylpyridine	20.1	93	66	-	-	
16	benzofuran	24.7	118	89	0.5	2.4	23.4 – 25.9
17	indan	25.2	117	91	0.4	1.7	
18	indene	26.2	116	89	0.3	1.5	25.9 – 28.0
19	2-methylbenzofuran	28.8	131	103	1.3	6.1	28.0 – 30.0
20	2-methylindene	30.5	130	115	1.3	6.3	30.0 – 31.7
21	naphthalene-D_8	32.1	136	108	1.2	5.7	31.7 – 34.0
	naphthalene	32.1	128	102	1.2	5.7	
22	benzothiophene	32.5	134	89	0.9	4.5	
23	2,3-dimethylbenzofuran	33.0	146	131	1.3	6.0	
24	quinoline	35.1	129	102	6.3	30.4	34.0 – 38.5
25	2-methylnaphthalene	36.3	142	115	1.2	5.5	
	2-methylbenzothiophene	36.3	147	115	-	-	
26	5-methylbenzothiophene	36.7	147	115	-	-	
27	3-methylbenzothiophene	36.8	147	115	1.0	n.d	
	1-methylnaphthalene	36.9	142	115	1.2	5.7	
28	2-methylquinoline	37.1	143	128	6.6	31.8	
29	indole	39.3	117	90	3.6	17.4	38.5 – 41.0
30	2,5-dimethylbenzothiophene	40.0	162	147	-	-	
31	4-methylquinoline	40.3	143	115	-	-	
	1,3-dimethylnaphthalene	40.4	156	141		22.3	
32	2,4-dimethylquinoline	41.8	157	142	5.7	27.1	41.0 – 43.5
33	acenaphthene	42.8	154	176	4.9	23.4	
34	dibenzofuran	44.2	168	139	4.2	20.0	43.5 – end

n.d. = not determined; r_t = retention time

Table 6: Data for the assessment of best quantification method for selected compounds: linear correlation coefficient and variation from nominal value.

compound	external calibration		internal calibration		standard addition[a]		MHE[a]	
	R^2	variation from nominal value	R^2	variation from nominal value	R^2	variation from nominal value	R^2	variation from nominal value
benzene	0.998	-6 %	0.999	1 %	0.942	5 %	1.000	6 %
toluene	0.998	-7 %	0.999	0 %	0.930	36 %	1.000	3 %
naphthalene	0.995	-4 %	1.000	0 %	0.978	73 %	0.994	16 %
indan	0.997	-10 %	0.991	-11 %	0.981	-34 %	0.996	3 %
acenaphthene	0.975	1 %	0.891	26 %	0.972	-7 %	0.974	18 %
furan	0.998	-6 %	1.000	1 %	0.970	3 %	0.998	4 %
benzofuran	0.992	-9 %	0.991	-7 %	0.985	13 %	0.997	7 %
dibenzofuran	0.983	-6 %	0.974	-6 %	0.983	96 %	0.971	-4 %
pyrrol	0.986	-11 %	0.984	-4 %	0.983	-8 %	0.998	1 %
pyridine	0.994	-7 %	1.000	0 %	0.948	-46 %	0.994	6 %
indole	0.976	-9 %	0.983	-2 %	0.910	52 %	0.932	0 %
quinoline	0.945	-10 %	0.951	-11 %	0.904	-30 %	0.923	8 %
thiophene	0.996	-13 %	0.999	1 %	0.931	57 %	0.999	9 %
benzothiophene	0.973	4 %	0.976	15 %	0.908	-3 %	0.994	15 %

[a] evaluation using direct peak area, no correction by internal standard

The comparison of quantification methods investigated here showed good results for all methods investigated. Only the method of standard addition led, in comparison to other methods investigated, higher deviation from nominal value. This deviation may be decreased using internal standards. Therefore, in the choice of analytical method, practical reasons have to be considered. The disadvantage of the MHE method is the necessity of long time (~400 min per sample), which is not practicable for analyses in routine.

The method using the correction by internal standards is favoured. Drifting of the instruments sensitivity within the time of the sequence as well as the advantage of avoiding influences of the matrix is minimized. Until now there are only few labelled compounds available, which may be used as internal standards. Therefore, it was of high importance to find out which of the internal standards available are useful for the broad number of compounds. Data of internal standard method shown in Table 6 are a result of using toluene-D_8 for toluene and benzene; naphthalene-D_8 for naphthalene, indan, acenaphthene; furan-D_4 for furan, benzofuran and dibenzofuran; pyridine-D_5 for pyrrol, pyridine, indole and quinoline; thiophene-$D_{2,5}$ for thiophene and benzothiophene. Linear correlation coefficients for compounds and their deuterated analogs showed highest correlation and lowest deviation from nominal value. Using the ratio of compounds with different sensitivities towards the detector, the error increases, especially if internal standard concentration is not in the range of compounds concentration. However, the labelled monocyclic compounds are useful even for the quantification of 3-ring compounds, but an analytical error of 15 % has to be accepted, when such a broad number of compounds has to be analysed.

4.1.2.2 Discussion of the Headspace-GC-MS method

The developed method allows the analysis of a broad number of tar oil contaminants, homocyclic as well as heterocyclic compounds. The method, which is routinely used for BTEX-analysis, is easily enlargeable on volatile homocycles and NSO-heterocyclic compounds. Therefore, information about a broad number of contaminants in groundwater is reachable in only one run of measurements.

In contrast to other methods, where sample preparation has to be performed, this method has the advantage that groundwater samples can be measured directly after addition of salt and internal standards. Losses of volatile compounds due to sample preparation are excluded. The usage of labelled standard compounds is of high importance. Monocyclic standards commercially available yielded acceptable results even up to 3-ring compounds.

4.1.2.3 Application of the Headspace-GC method for groundwater analyses

The groundwater of three tar oil contaminated sites was analysed for the occurrence of 1-ring heterocyclic compounds (data of 2- and 3-ring compounds are not presented here, because they are shown in detail in chapter 4.2).

Only a limited number of 1-ring heterocyclic contaminants were detected at the Castrop-Rauxel and the Wülknitz site in the groundwater of the highest contaminated wells, while none of these compounds were present, even in the highest contaminated groundwater analysed of the Düsseldorf-Flingern site. In contrast, in all of the investigated samples BTEX were present (quantitative data are given in Table 25, appendix).

Within the monoaromatic heterocycles, only N- and S-heterocycles were found. The group of N-heterocycles was represented by 2-methyl-, 3-methyl- as well as 4-methylpyridine, while the non-substituted compounds pyridine and pyrrol were absent. At the Castrop-Rauxel site 2-methylpyridine was present in highest concentrations (576 µg/L) within the methylisomers, followed by 3-methylpyridine with 94 µg/L and at least 58 µg/L 4-methylpyridine, while at the Wülknitz site the 4-methylanalog was present in highest concentration, followed by the 3-methyl- and at least 2-methyl-isomer.

Monocyclic S-heterocycles were only detectable at the Wülknitz site. The presence of 2-methyl-, 3-methyl- and 2,5-dimethylthiophene was indicated, but concentrations were found near the limit of detection. The non-substituted parent compound thiophene was absent. O-Heterocyclic compounds such as furan and methylfurans were not determined at any of the three sites.

Although the monocyclic heterocyclic compounds exhibit higher water solubility and therefore may be dissolved even to higher extent into the water phase, their minor presence in groundwater was surprising as they are described in literature as typical tar oil components (Lang and Eigen, 1967). In accordance to our findings, Stuermer et al. (1982) found a broad number of methylsubstituted pyridines and several alkylthiophenes in the groundwater of a coal gasification site. Also Johansen et al. (1997b) detected monocyclic N- and S-heterocycles in the groundwater of three tar oil contaminated sites in similar concentrations as has been found in this study. In addition to alkylated pyridines also pyrrol and 1-methylpyrrol were detected. However, all monocyclic N-heterocyclic compounds detected were only present in a restricted number of groundwater samples. The same was true for methyl- and dimethylthiophenes, while thiophene was found more distributed, but in concentrations lower than LODs of the presented method.

The results show that monoaromatic heterocyclic are of minor relevance for the assessment of groundwater at tar oil contaminated sites because they are only present in highest contaminated regions. This observation is in contrast to their physicochemical characteristics. As they are more polar with higher water solubility and showed less sorptive characteristics than monoaromatic homocycles or 2-ring heterocycles, it was expected to determine these compounds even downstream the plume. Therefore, the minor presence and low distribution may be due to low concentrations within the contamination source or high microbial degradative potential towards these compounds. Kuhn and Suflita (1989), who studied the anaerobic degradation of monoaromatic heterocycles, found methylated analogs less amenable than non-substituted heterocycles. This observation is in accordance to data from the field sites where pyridine was not determined, while methylsubstituted pyridines reached high concentrations.

4.2 Tar oil contaminated sites: Relevance and fate of heterocyclic compounds

4.2.1 Site-directed analyses: Relevance of heterocyclic compounds

The groundwater of three tar oil contaminated sites, the coke manufacturing site at Castrop-Rauxel, the gasification site at Düsseldorf-Flingern, and the wood impregnation site at Wülknitz, was analysed for the occurrence of NSO-heterocyclic compounds with special emphasis on quinoline compounds. The following approaches were performed:

- Estimation of mass balance of NSO-heterocyclic and selected homocyclic compounds in the field site to study the presence of natural attenuation.
- Investigation of the distribution of different compounds of contaminants over the field sites to evaluate correlations within the group of heterocycles.
- Comparison of heterocycles to PAHs and determination of contaminants of relevance downstream the source.

4.2.1.1 Coke manufacturing site at Castrop-Rauxel

The analyses of groundwater samples of wells in the testfield at the Castrop-Rauxel site gave a lot of information about the heterocyclic compounds present, their distribution and occurrence near the source and downstream the plume, which may be discussed in context to microbial degradative NA-processes.

The testfield (40 × 36 m) with three lines located nearly in vertical to the groundwater flow, formed by 5 three-level-wells, allowed to study the distribution of compounds in a manageable region (Figure 5). Furthermore, the well structured network of wells allowed to form mass balances, to get information about the decrease of contaminants over a distance of 36 m. All analytical data of sampling in March 2005 are summarized in Table 26 and 27.

Groundwater analyses: Mass balance of contaminants

The mass load and mass balance of contaminants over the area of the testfield was carried out in the following way: Average data from the 5 wells with its 3 depth from the southern line of the testfield (entrance) are compared to data from the northern line (exit) in a distance of about 36 m. The line of wells in the middle of the testfield was not considered.

Calculations of flow rates were done by Breul (2004) taking into consideration the differences in K_f-values, filter units and hydraulic gradients all over the site and an increase in amounts of groundwater due to rainfalls (~151 m^3/a), which let to 2688 m^3/a of groundwater flowing into the southern line (size 40 × 5 m) and 2839 m^3/a leaving the northern line of the testfield (Figure 12).

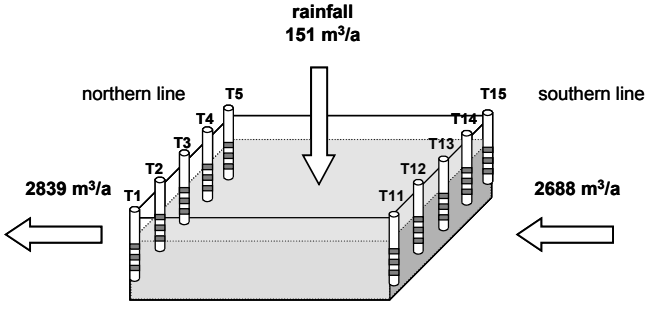


Figure 12: Estimation of the mass flow in the quaternary aquifer of the testfield (according to Breul, 2004).

For the calculation of mass flow of contaminants the volume flowing through the axes of the testfield was multiplied with the average of contaminants concentration of the 5 wells with 3 depths. In general high differences in reduction in concentration of compounds within the distance of 36 m in the testfield were detected. Data are summarized in Table 7.

The amounts of several heterocyclic and homocyclic compounds are comparable to amounts of naphthalene, the best water soluble EPA-PAH. EPA-PAHs form up to 9 kg/a, while the major part is formed by naphthalene (7.8 kg/a). Further compounds found in highest concentrations are: 3,4-dihydro-2(1H)-quinolinone (2.8 kg/a), 2(1H)-quinolinone (5.0 kg/a), 1(2H)-isoquinolinone (1.8 kg/a), 2-methylnaphthalene (1.3 kg/a), benzofuran (2.4 kg/a), 1-methylnaphthalene (1.8 kg/a), indene (7.5 kg/a), benzothiophene (2.8 kg/a), 2-methylbenzofuran (1.1 kg/a) and indan (1.9 kg/a).

N-Heterocycles. All quinoline compounds investigated are mostly eliminated over a distance of 36 m within the testfield. However, some differences were determined.

Quinoline is decreased in higher extent than its isomeric analog isoquinoline, although quinoline is present at higher concentration in the first line. Similarly, higher decrease of 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone, the two quinoline compounds entering the testfield in highest amounts, was found than the decrease of the isomeric compounds 1(2H)-isoquinolinone and 3,4-dihydro-2(1H)-isoquinolinone.

A difference in the tendency of elimination of the parent compound quinoline and its hydroxylated analogs could not be observed, because of the generally high decrease in the range of 99.9 %. There were no differences in decrease of methyl- as well as dimethylquinoline isomers detectable. A minor difference was determined in decrease of 4-methylquinoline and its hydroxylated analog 4-methyl-2(1H)-quinolinone, elimination of 4-methylquinoline was higher.

S- and O-Heterocycles. Studying the decrease of O-heterocycles present, benzofuran, present in clearly higher concentrations than the other O-heterocycles, shows higher decrease (94 %) in comparison to its methyl- and dimethylsubstituted analogs (decrease of 69-80 %) as well as 3-ring analog dibenzofuran (decrease of 89 %).

The S-heterocyclic compound benzothiophene showed a decrease of about 83 %, while the decrease of 3-methylbenzothiophene was only about 77 %. Hence in both cases the decrease of methylsubstituted compounds was less, although starting concentrations were lower than those of non-substituted compounds.

Homocycles/PAHs. A high difference in decrease was observed for the isomeric methylnaphthalenes - 1-methyl- and 2-methylnaphthalene -, which entered the testfield in similar concentrations. The reduction in concentration of 2-methylnaphthalene (97 %) was obviously higher than the decrease of its analog 1-methylnaphthalene (87 %). The parent compound naphthalene decreased at about 87 %, however, its input was about a factor 4 higher than the input of methylnaphthalenes.

Decrease of the homocyclic compounds indene and indan was determined with 85 and 75 % in the lower range of decrease of investigated compounds. Furthermore, the 3-ring PAHs acenaphthene and fluorene, generally present in low concentrations, were eliminated only at about 78 and 69 % within the testfield.

Table 7: Decrease in mass load of heterocyclic and homocyclic compounds within the Castrop-Rauxel testfield over a distance of 36 m.

compound	concentration in line 3 [µg/L]+	decrease [%]	mass load entering the testfield (40 × 5 m) [kg/a]
N-Heterocycles			
quinoline	0.08	99.9	0.28
isoquinoline	0.15	98.2	0.02
1-methylisoquinoline	0.03	99.7	0.03
3-methylisoquinoline	0.02	99.7	0.02
2-methylquinoline	0.10	99.9	0.23
4-methylquinoline	0.19	99.9	0.45
6-methylquinoline	0.02	99.8	0.03
2(1H)-quinolinone	0.34	100	4.96
1(2H)-isoquinolinone	14	97.7	1.76
4(1H)-quinolinone	0.02	99.7	0.02
7-isoquinolinol	0.03	99.6	0.02
3(2H)-isoquinolinone	<0.004	100	0.02
3,4-dihydro-2(1H)-quinolinone	0.07	100	2.75
3,4-dihydro-1(2H)-isoquinolinone	0.05	99.5	0.03
2-methyl-6-quinolinol	<0.003	100	0.03
4-methyl-2(1H)-quinolinone	3.1	98.7	0.68
6-methoxyquinoline	0.01	99.8	0.02
1-methyl-2(1H)-quinolinone	0.72	94.1	0.04
2-methyl-4(1H)-quinolinone	0.66	90.3	0.02
2,4-dimethylquinoline	0.24	99.8	0.43
2,6-dimethylquinoline	0.02	99.8	0.03
1,2,3,4-tetrahydroquinoline	<0.013	100	0.01
7-hydroxy-4-methyl-2(1H)-quinolinone	0.06	99.0	0.02
4-hydroxy-1-methyl-2(1H)-quinolinone	0.25	91.8	0.01
sum of N-Heterocycles	21	99.5	10.7
S-Heterocycles			
benzothiophene	169	82.6	2.76
3-methylbenzothiophene	51	77.4	0.64
sum of S-Heterocycles	220	81.6	3.40
O-Heterocycles			
benzofuran	49	94.0	2.45
2-methylbenzofuran	80	79.6	1.11
methylbenzofuran (isomer)	55	69.0	0.50
2,3-dimethylbenzofuran	25	68.0	0.22
dibenzofuran	7.0	89.4	0.19
sum of O-Heterocycles	215	86.4	4.47
PAHs/Homocycles			
naphthalene	344	87.4	7.78
fluorene	5.4	68.8	0.05
acenaphthene	30	77.8	0.38
sum of EPA-PAHs++	416	86.4	8.69
1-methylnaphthalene	84	86.6	1.77
2-methylnaphthalene	14	97.1	1.35
1,3-dimethylnaphthalene	6.3	87.4	0.14
indene	391	85.2	7.47
indan	173	74.6	1.94
1-indanone	0.2	99.9	1.27
1-naphthol	<0.4	98.1	0.05

+average data of line 3 are given. ++data of the engineer office Dr. Wessling Beratende Ingenieure, Altenberge, Germany. In those cases, where the analytics indicated the absence of a compound, half of the compounds detection limit was used for the calculation of mean concentrations, which were used for the calculation of mass loads. Using this procedure, the presented data show the lowest decrease in concentration over the field site in contrast to other calculation approaches using the parameter "absence of compound".

Hydroxylated compounds. In addition to hydroxylated quinoline compounds as mentioned above, some additional hydroxylated compounds were found in the plume. 1-Indanone and 1-naphthol were eliminated completely within a distance of 36 m, although a high amount of 1-indanone (1.3 kg/a) entered the testfield.

Distribution of compounds within the testfield

In addition to the mass flow analyses the type of distribution of the compounds in the testfield was analysed in detail to evaluate possible correlations within the heterocycles.

S- and O-Heterocycles. The distribution of selected compounds like benzofuran, 2-methylbenzofuran and benzothiophene in the testfield is shown in Figure 13. Distribution all over the testfield is obvious for the three compounds shown, which are generally present in a similar range of concentration. An increase in concentration of the three compounds was found with increasing depth. However, the patterns in distribution of these three compounds are inhomogeneous: while benzofuran is present in higher or similar concentrations in line 1 (well T11-15) than 2-methylbenzofuran and benzothiophene, it is of less relevance in line 3. In contrast to benzofuran, which shows low concentrations in the wells on the right side of the testfield (T15 and T10), the high decrease in concentration in these wells was absent for 2-methylbenzofuran and benzothiophene.

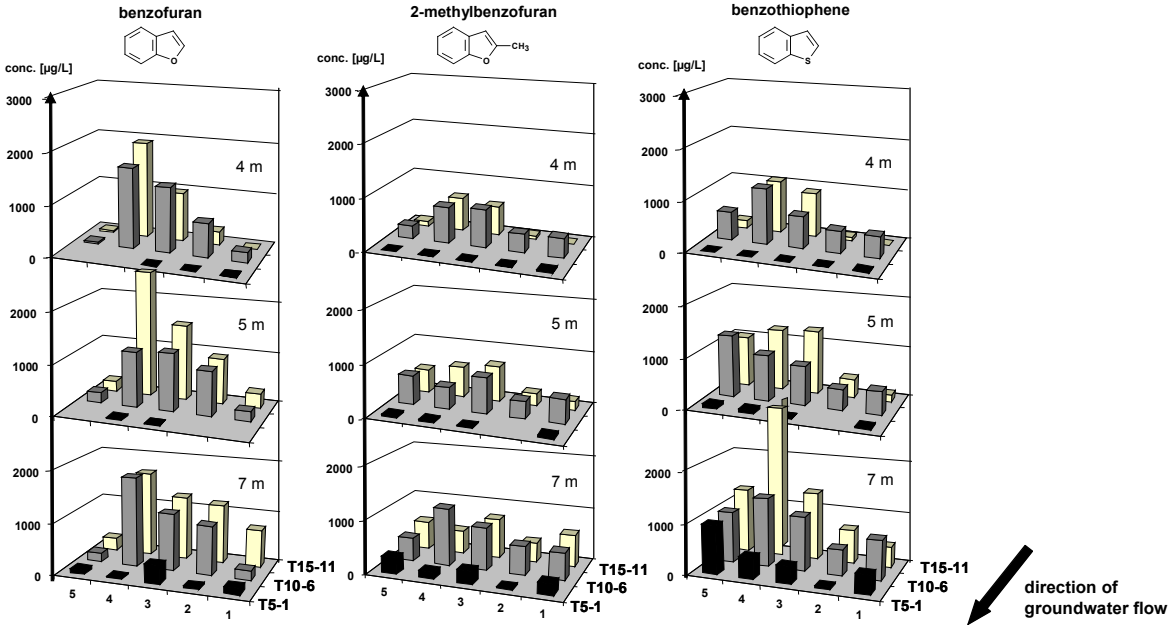


Figure 13: Distribution of selected heterocyclic compounds benzofuran, 2-methylbenzofuran and benzothiophene in groundwater of the testfield with its 15 three-level wells (4, 5, 7 m depth) in Castrop-Rauxel.

Quinoline compounds. In contrast to the S- and O-heterocycles, quinoline, isoquinoline and hydroxylated analogs were found to be less evenly spread over the site and showed higher variances in concentration. They were mostly present on the right side of the testfield, in well T13-15 and T8-9 (Figure 14). In general, low concentrations of quinoline (460 µg/L in maximum) were present accompanied by higher ones of 2(1H)-quinolinone (up to 5200 µg/L), and 3,4-dihydro-2(1H)-quinolinone (up to 2300 µg/L). An existing correlation was shown by the following points: largest concentrations were found in T13-15 and T8-9 and a decline in concentration was observed with groundwater flow, leading to minor

concentrations downstream (T1-5). In those wells where quinoline was absent, only small amounts of 2(1H)-quinolinone were observed. Highest amounts of 2(1H)-quinolinone occurred in the presence of high amounts of quinoline. 3,4-Dihydro-2(1H)-quinolinone was detected in those wells where the highest concentrations of quinoline and 2(1H)-quinolinone were found.

The observations at the field site concerning isoquinoline and its hydroxylated product 1(2H)-isoquinolinone are similar to the data obtained for quinoline compounds. Like quinoline, the parent compound isoquinoline is present in lower concentrations than the hydroxylated analog. 3,4-Dihydro-1(2H)-isoquinolinone was found only in minor amounts, less than isoquinoline itself. Generally, isoquinoline as well as 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone were present in concentrations 5-10 times lower than quinoline compounds.

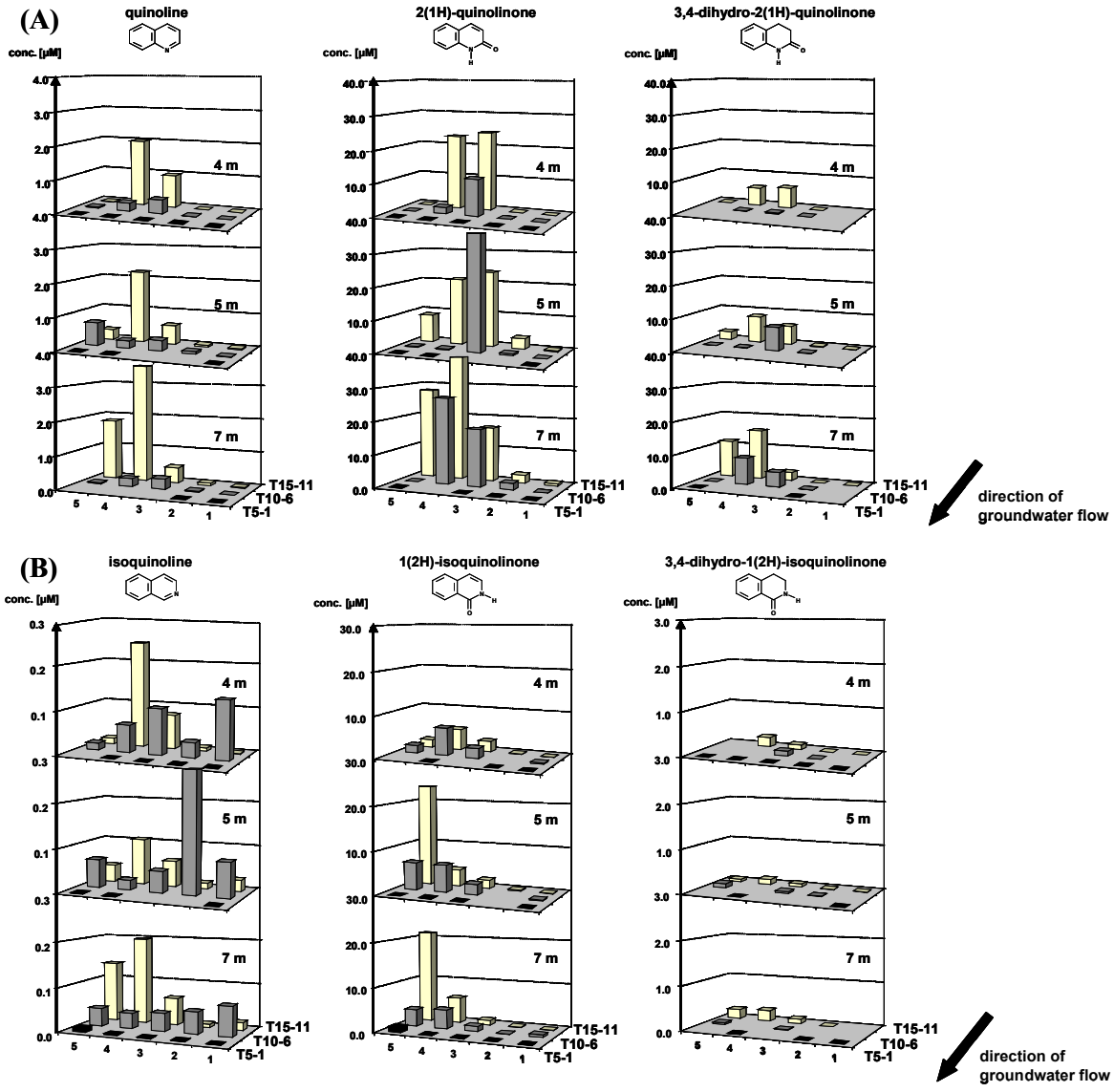


Figure 14: Distribution of (A) quinoline, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone and (B) isoquinoline, 1(2H)-isoquinolinone and 3,4-dihydro-2(1H)-quinolinone in the groundwater of the testfield with its 15 three-level wells (4, 5, 7 m depth) in Castrop-Rauxel.
Note differences in y-axis! For a better comparison of parent and metabolite molar concentrations are given here: quinoline and isoquinoline: 1 µM = 129 µg/L; 2(1H)-quinolinone and 1(2H)-isoquinolinone 1 µM = 145 µg/L; 3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone: 1 µM = 147 µg/L.

Distribution as well as range of concentration of 4-methylquinoline was found to be similar to quinoline. As has been shown for 2(1H)-quinolinone and 1(2H)-isoquinolinone the amount of 4-methyl-2(1H)-quinolinone exceeded the amount of 4-methylquinoline. The same is true, even in higher extent, when comparing the sum of methylsubstituted parent compounds to the sum of their hydroxylated analogs (Figure 48).

Comparison of different groups of compounds

To find out differences in decrease with groundwater flow direction which may give good indications for NA, the group of heterocyclic compounds was compared to EPA-PAHs (data kindly provided from the engineer office Dr. Wessling Beratende Ingenieure, Altenberge, Germany).

As shown in Figure 15 the ratios of NSO-heterocycles/PAHs and NSO-heterocycles/acenaphthene as well as single groups of heterocycles versus PAHs or acenaphthene were formed as median values of the three lines of wells in the Castrop-Rauxel testfield. Acenaphthene was chosen as highly persistent compound and therefore as worst-case compound (Zamifirescu and Grathwohl, 2001).

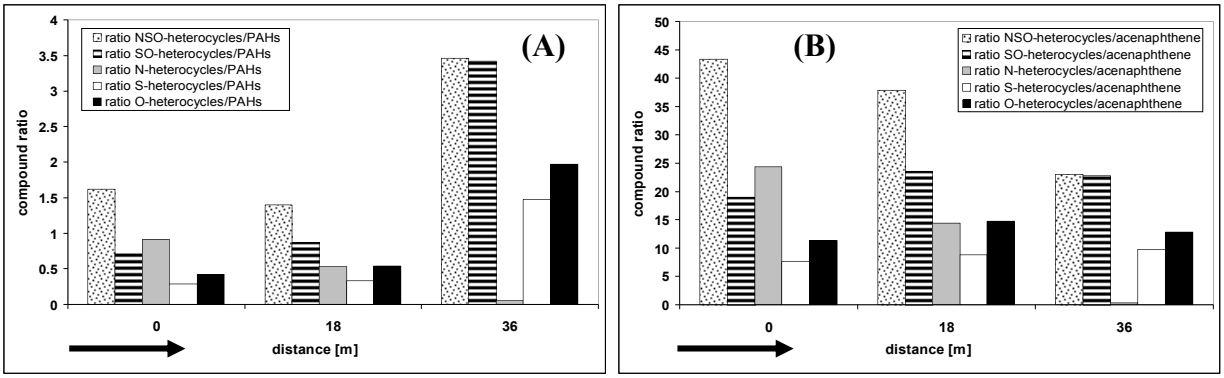


Figure 15: Ratio of NSO-heterocycles and single groups of heterocycles to (A) the group of EPA-PAHs as well as (B) acenaphthene at the Castrop-Rauxel site. The bars represent median values of concentrations of the lines of wells; the arrow marks the direction of groundwater flow.

In all of the three lines of wells investigated NSO-heterocycles are present in high amounts, even in higher amounts than EPA-PAHs. The ratio of NSO-heterocycles/PAHs increases with the groundwater flow direction. However, this conclusion has to be analysed in detail: especially S- and O-heterocyclic compounds gain high importance with distance from the source, while the group of N-heterocycles decreased in the flowline. In the first line of wells N-heterocycles represent the group of highest concentrations. The ratio of NSO-heterocycles is about **1:0.3:0.5**, while the ratios in the following lines (line 2: **1:0.6:1** and line 3: **1:30:40**) are shifted to the group of S- as well as O-heterocycles.

The importance of the heterocyclic compounds is confirmed, when comparing them with the highly persistent PAH acenaphthene. The ratio of median concentrations of the S- and O-heterocycles versus acenaphthene is about the same in all of the three lines of wells. Therefore, the data clearly show that S- and O-heterocyclic compounds are of identical relevance downstream as known for acenaphthene.

The presented data resulted from the study of the well-suited testfield and flow distance of 36 m. However, when including the data from wells (T18 and T19, see Table 28) located 100 m downstream from line 3, the group of heterocycles generally is found of less

relevance. Much higher concentrations of PAHs lead to lower ratios. Even S- and O-heterocycles are found in lower concentrations relative to PAHs. Surprisingly, naphthalene and acenaphthene were the PAHs which were still present, although naphthalene is known as being degradable. Furthermore, the ratio of NSO-heterocycles was **1:5:4**, in contrast to **1:30:40** in line 3 of the testfield. It has to kept in mind that these discrepancies in ratios, found in the testfield and those obtained over such a long distance, may be influenced by hot spots of contamination over the distance of 100 m. This may also explain the high concentration of naphthalene as well as the higher amounts of N-heterocycles relative to S- and O-heterocycles found in line 3 of the testfield.

Identification of quinoline compounds by LC-NMR

For the identification of highly polar compounds within the tar oil contaminated groundwater online as well as offline NMR-techniques supported by results from MS-measurements were performed (M. Elend and A. Preiß; Fraunhofer Institute for Toxicology and Experimental Medicine, ITEM, Hannover).

A broad number of hydroxylated as well as hydroxy-methylated quinoline compounds was detected by the HPLC-MS-MS chromatogram as well as DAD-spectra, analysing the extract of the groundwater sample T13 (5 m depth). Because of the limited commercial availability of methylquinoline isomers and methyl-2(1H)-quinolinones, only few of these compounds could be assigned to a special isomeric structure. Furthermore, the separation by HPLC is limited when a broad number of isomers is present. However, a concrete identification of isomeric structure may be of interest since the position of a methylsubstituent is known to influence biodegradation margely.

Figure 16 (A) shows a section of the obtained on-flow H-NMR chromatogram of the mentioned groundwater sample. The first four eluting compounds, the compounds with the highest polarity as well as high concentrations within the groundwater, could be identified as 2(1H)-quinolinone, 1(2H)-isoquinolinone, 4-methyl-2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone. Between 81 and 98 min 3-, 6-, 7- and 8-methyl-2(1H)-quinolinone eluted successively, while the less polar 6- and 7-methyl-3,4-dihydro-2(1H)-quinolinone co-eluted at 120 min (concrete data about ^{1}H-NMR as well as ^{13}C-NMR-shifts are presented in Table 29). In Figure 16 (B) extracted rows of the NMR chromatogram are shown which display the ^{1}H NMR spectra of the early and the late eluting quinolinones. Row 86 represents an example showing strongly increases in the selectivity by LC-NMR: the two methyl-3,4-dihydro-2(1H)-quinolinones, which co-elute in the chromatographic axis, are well resolved in the NMR-dimension.

The analysis of the rows allowed structural proposals for isomers of several formerly unknown compounds. These results were further confirmed by offline NMR- and MS-measurements. Some compounds which, due to the lack of sensitivity, were not visible in the on-flow chromatogram (detection limits are in the lower µg-range), could be identified using the later procedure.

Generally, hydroxylated quinoline compounds were found as the most polar compounds within high concentration in the tar oil contaminated groundwater. A broad number of methylsubstituted 2(1H)-quinolinones was determined in the groundwater. Furthermore, reduced species i.e. methyl-3,4-dihydro-2(1H)-quinolinones were found within the components of the tar oil contamination. These compounds were present in the groundwater in concentrations in the upper µg/L-range or even higher. Interestingly only those

hydroxylated quinolines were observed, which carry the hydroxysubstituent in position 2. In contrast, methylsubstituted isoquinolinone isomers were not detected.

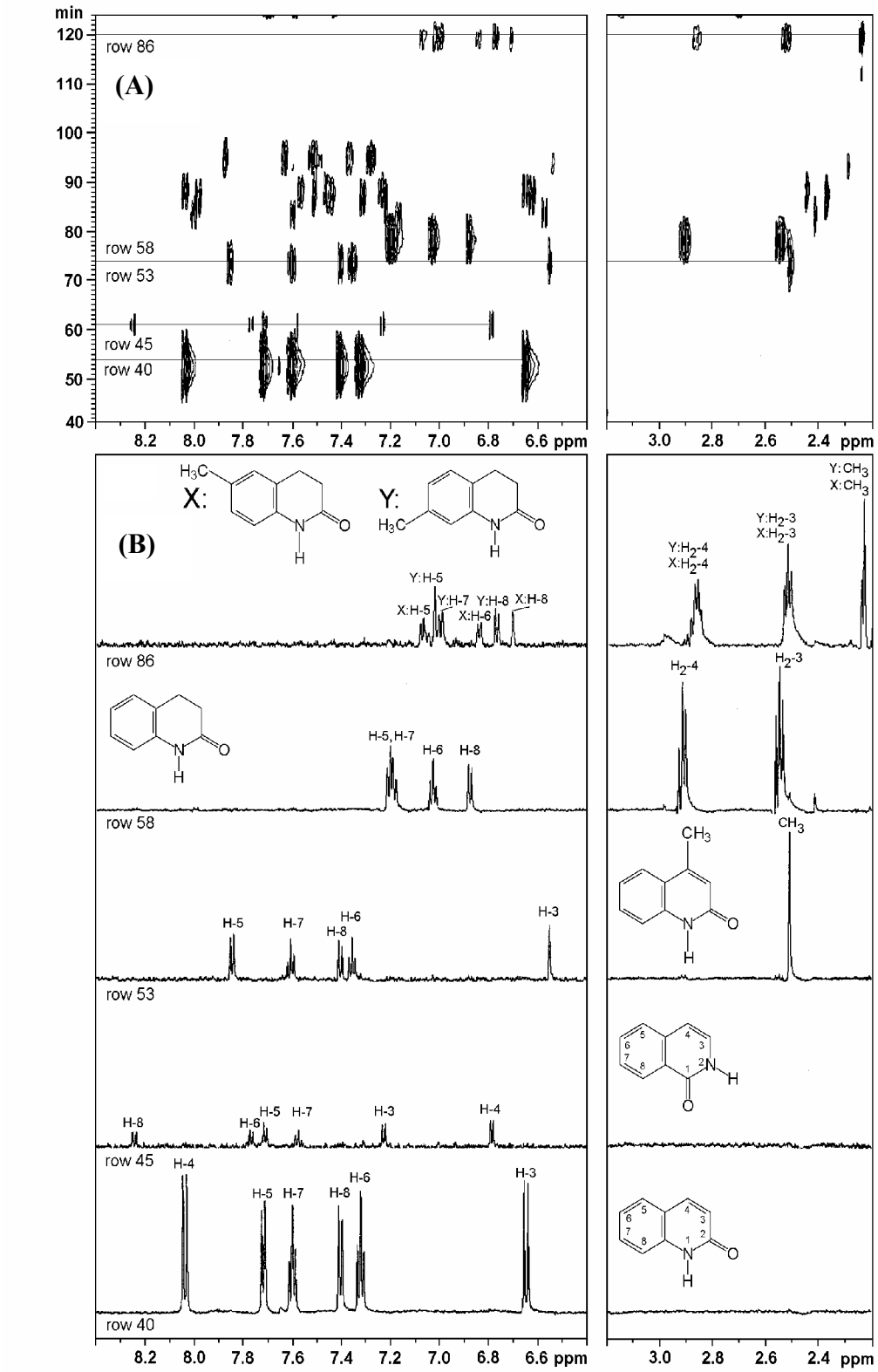


Figure 16: (A) Sections of the on-flow NMR chromatogram of an enriched groundwater sample T13 (5 m depth) of the Castrop-Rauxel site. (B) Rows extracted from the NMR chromatogram.

4.2.1.2 Gasworks site at Düsseldorf-Flingern

Groundwater of 14 wells with up to 5 depths at the Düsseldorf-Flingern site was analysed. The wells form 5 lines down the groundwater flow and describe the plume over a distance of 25, 50, 85 and 130 m (Figure 17).

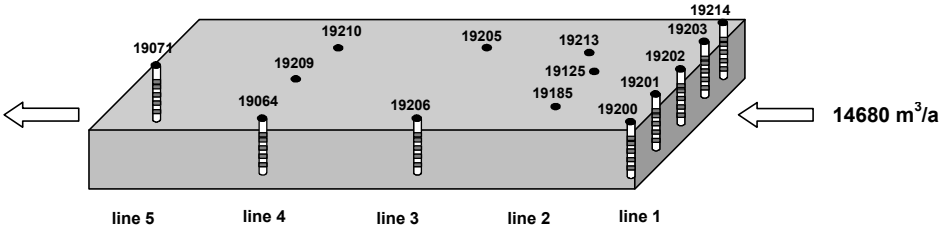


Figure 17: The gasworks site Düsseldorf-Flingern; 5 lines of wells are formed.

The concentration of organic contaminants in the groundwater at the field site are listed in Table 30 and 31. Groundwater samples were taken in October 2005 and were analysed for NSO-heterocycles and homocycles. Special emphasis was set on quinoline compounds. A comparison of compounds distribution was focused to work out hints for microbial degradation.

Ground water analyses: Mass balance of contaminants

The mass balance at the Düsseldorf-Flingern site was investigated using four lines of wells as balancing lines. Line 1 formed by 5 wells with 5 depths was used as starting point. These wells include an area of 35 × 3.5 m, resulting in an amount of 14680 m^3/a flowing into the field site. The contaminants mass flow concentration was compared with the calculated amounts flowing through line 4 (formed by 3 wells) and line 5 (only one well). Dilution over the field site due to rainfalls was not considered, because main parts of the field site are overbuilt or exhibit an asphalted surface. Amounts entering the field site and the decrease over the distance of 25, 85 and 130 m are summarized in Table 8.

Of the investigated compounds, only a few are present in the first line of wells analysed in high amounts in the kg/a-range. These are beneath the PAHs acenaphthene (8.5 kg/a), fluorene (3.3 kg/a) naphthalene (6.1 kg/a) and 1-methylnaphthalene (3.4 kg/a), some heterocycles like dibenzofuran (3.3 kg/a), benzothiophene (1.2 kg/a) and 3-methylbenzothiophene (1.0 kg/a).

N-Heterocycles. In general, no N-heterocyclic compound was detected in amounts comparable to PAHs. Amounts of these were about a factor of 10-1000 lower. Highest concentrations of quinoline compounds were found in the wells with lowest depth. Quinoline was mainly present in the first line of wells in low concentrations up to 1.6 µg/L and enters the field site only in an amount of 1.6 g/a. Within a distance of about 25 m the concentration decreased to a level near the limit of detection. In the fourth line quinoline was no more detectable. In comparison its isomeric compound isoquinoline was found in a concentration up to 8 µg/L in the first line and enters the field site in an amount 5 times higher (8.8 g/a) than quinoline. Its decrease over the field was minor and it was detectable in all wells, even in a distance of 130 m from the highest contaminated line studied. However, concentrations were generally low, within the lower µg/L-range. In contrast the hydroxylated compounds 2(1H)-quinolinone and 1(2H)-isoquinolinone were found more widespread and in higher

concentrations than their parent compounds. 1(2H)-isoquinolinone was present in higher amounts (286 g/a) than 2(1H)-quinolinone (17 g/a). Furthermore, the hydroxylated and reduced compounds 3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone were determined but in lower amounts.

Highest concentration of 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone were detected in well 19201, 6.5 m depth (14.4 and 5.4 µg/L, respectively). Even in a distance of 25 m in line 2 concentration of 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone only decreased by 53 and 60 %, respectively, while decrease of quinoline was higher, up to 86 %. The same was true for the isomeric analogs: the decrease in concentration of isoquinoline over a distance of 25 m was about 72 % of the initial concentration, while the decrease of 1(2H)-isoquinolinone reached only about 53 %. However, decrease of 3,4-dihydro-1(2H)-isoquinolinone occurred in a lower range and was only about 38 %.

1(2H)-Isoquinolinone (maximum 137 µg/L) was generally present in higher concentrations than 2(1H)-quinolinone (maximum 14 µg/L). For the further reduced chemical species the other way round was determined: 3,4-Dihydro-2(1H)-quinolinone was found in higher concentrations (5.4 µg/L in maximum) than 3,4-dihydro-1(2H)-isoquinolinone, which was only present in concentrations near the limit of detection.

Downstream in well 19209, no quinoline was detectable, while 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone as well as isoquinoline, 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone were present. Well 19071 is the one most downstream from the original contamination source. Even in this distance all the isoquinoline analogs, isoquinoline, 1(2H)-isoquinolinone, 3,4-dihydro-1(2H)-isoquinolinone, were detectable in minor amounts, while 2(1H)-quinolinone was the only quinoline analog present.

A broad number of methylquinolines was detected all over the field site. Out of the isomers analysed, 2-methylquinoline was present in higher amounts than the other methyl-isomers, in line 1 (188 in contrast to 2-48 g/a), as well as in the lines downstream. Looking at the ratios of different methylquinoline isomers/2-methylquinoline, there is no tendency detectable of decreasing or increasing ratio with the groundwater flow direction. However, out of all isomers 2-methylquinoline was the only one, which was still detectable in groundwater from well 19071, the well located most downstream. As has been observed for the parent compounds quinoline and isoquinoline, 4-methylquinoline was present in lower concentrations than its hydroxylated analog 4-methyl-2(1H)-quinolinone.

Dimethylquinolines (2,4- and 2,6- analogs) were found to be present in the same or even higher amounts than methylquinolines, 241 and 74 g/a, respectively, enter the field site. However, their decrease over the distance of the site is found to be even higher than decrease of methylquinolines.

Several 3-ring N-heterocyclic compounds were found: Acridine was only detected in few wells in the first (1.8 g/a) and second line. Concentrations generally were low (up to 4 µg/L), while its hydroxylated analog 9(10H)-acridinone was found to be more widespread in some higher concentrations up to 16 µg/L. Its amount entering the field was found about 69 g/a and therefore a factor 38 higher than the amount of the non-substituted parent compound. 9(10H)-Acridinone was still detectable in the fourth line of wells, 85 m distance from the highest contaminated line. Carbazole was detected in most wells in the first and second line and only in few wells in the third and forth line. Its amount was found about 402 g/a and is therefore much higher than the amount of its analog acridine. There was no indication for the occurrence of the hydroxylated analog of carbazole. Also 6(5H)-phenanthridinone was determined all over the field and reached 41 µg/L in the fourth line, while the non-substituted parent compound phenanthridine was absent.

Table 8: Decrease in mass load through a section of 35 × 3.5 m of heterocyclic and homocyclic compounds within the Düsseldorf-Flingern field site (line 2, 4 and 5) over a distance of 25, 85 and 130 m.

compound	concentration in line 5 [µg/L]	decrease [%] line 1-5	decrease [%] line 1-4	decrease [%] line 1-2	mass load entering the field site [g/a]
N-Heterocycles					
quinoline	<0.0006	99.7	97.2	86.3	1.6
isoquinoline	0.020	96.5	84.6	77.2	8.8
1-methylisoquinoline	0.004	99.9	98.4	+	48
3-methylisoquinoline	0.006	98.7	92.2	95.1	6.4
2-methylquinoline	0.050	99.6	53.7	+	188
4-methylquinoline	<0.0004	99.8	92.4	77.1	1.6
6-methylquinoline	0.001	99.9	98.4	+	17
2(1H)-quinolinone	0.002	99.9	90.6	52.9	17
1(2H)-isoquinolinone	0.020	99.9	96.5	53.0	286
4(1H)-quinolinone	<0.0008	89.6	+	+	0.2
3,4-dihydro-2(1H)-quinolinone	<0.0008	99.9	60.7	60.2	4.5
3,4-dihydro-1(2H)-isoquinolinone	0.004	79.4	0	37.7	0.1
4-methyl-2(1H)-quinolinone	0.008	99.7	35.7	77.8	53
6-methoxyquinoline	<0.0003	59.5	13.6*	13.6*	0.01
1-methyl-2-quinolinone	0.020	85.6	+	+	2.4
1-methyl-2,4-quinolinol	<0.0006	99.6	+	+	1.0
2,4-dimethylquinoline	<0.0006	100	99.8	17.1	241
2,6-dimethylquinoline	<0.0006	100	99.8	47.6	74
carbazole	<0.04	99.9	93.8*	58.4	402
acridine	<0.24	90.6	5.8*	5.8*	1.8
9(10H)-acridinone	<0.24	97.7	68.5	37.0	69
6(5H)-phenanthridinone	<0.04	99.8	44.5	+	152
sum of N-Heterocycles	0.135	99.9	85.5	35.7	953
S-Heterocycles					
benzothiophene	3.0	96.9	50.8	60.9	1435
3-methylbenzothiophene	4.9	94.2	93.2	31.0	1258
methylbenzothiophene isomer 1	<0.08	99.9	87.9	56.8	484
methylbenzothiophene isomer 2	<0.08	99.9*	44.4*	44.4*	5.0
methylbenzothiophene isomer 3	<0.08	99.9*	35.1*	35.1*	3.4
dimethylbenzothiophene	65	+	84.8	+	14.5
dimethylbenzothiophene	<0.04	99.5	78.7	+	34
dibenzothiophene	<0.04	100	94.9	35.5	113
sum of S-Heterocycles	73	68.1	75.1	48.3	3363
O-Heterocycles					
benzofuran	<0.04	99.9	90.2	89.3	394
2-methylbenzofuran	<0.04	97.0	98.5	98.5	194
methylbenzofuran isomer 1	<0.04	98.1	+	69.7	9.2
methylbenzofuran isomer 2	<0.04	98.4	52.4	+	13
methylbenzofuran isomer 3	12	98.4	83.8	83.8	15
methylbenzofuran isomer 4	26	+	+	+	171
2,3-dimethylbenzofuran	<0.04	99.6	+	28.4	70
dimethylbenzofuran isomer	<0.04	99.9	90.8	0.6	253
dibenzofuran	0.6	99.8	98.4	92.1	3993
methyldibenzofuran isomer 1	14	+	45.8	9.5	68
methyldibenzofuran isomer 2	39	3.0	78.6	10.7	583
methyldibenzofuran isomer 3	65	+	73.6	0.2	479
sum of O-Heterocycles	156	63.3	85.4	68.5	6244
PAHs/Homocycles					
naphthalene	2.2	99.6	83.7	84.9	7335
fluorene	3.7	98.6	90.1	13.7	3953
acenaphthene	78	89.0	63.2	0	10335
sum of EPA-PAHs*	100	94.2	77.4	28.9	22616
1-methylnaphthalene	24	91.4	83.3	86.4	4155
2-methylnaphthalene	<0.04	100	98.9	99.5	546
indene	<0.04	100	96.7	67.3	1141
indan	8.7	99.8	+	0	522
1-indanone	<0.04	95.8*	57.9*	57.9*	4.1
1-naphthol	<0.04	100	87.8*	87.8*	12
2-naphthol	<0.04	98.0*	80.1*	80.1*	22
1-acenaphthenol	<0.02	98.8*	59.5*	7.4	13

+ increase in concentration; *data of EPA-PAHs were provided by Lars Richters, Stadtwerke Düsseldorf-Flingern. isomers are numbered if the position of substitution unknown; In those cases, where the analytics indicated the absence of a compound, half of the compounds detection limit was used for the calculation of mean concentrations, which were used for the calculation of mass loads. For compounds present in concentration near the detection limit even in the inflow the values of decrease in the mass balance may represent a clear underestimation. These cases are marked by (*).

S- and O-Heterocycles. A broad number of S- and O-heterocyclic 2-ring compounds was determined. When looking at the mass loads, benzothiophene, 3-methylbenzothiophene and dibenzofuran were found in the kg/a-range. Generally, in addition to the parent compounds, a broad number of methyl- as well as dimethylsubstituted analogs were present.

Benzofuran was mainly found in the highest contaminated wells in the first line. There were up to 5 methylbenzofuran isomers identified by DAD-spectra. However, concrete isomeric structure could not be determined. These isomers showed high recalcitrance and were still detectable in 130 m downstream. Also two dimethylbenzofuran isomers were widely distributed and were still detectable in concentrations up to 65 µg/L in 130 m distance of the first line of wells.

When looking at the S-heterocyclic compounds similar results were found. Even here methyl- and dimethylsubstituted analogs gain high importance. Benzothiophene was found in most wells near the source and was still present in the fourth and even fifth line of wells. In addition several methylated analogs (3 isomers) were detected. These were found in similar concentrations than benzothiophene, but furthermore were also detected in some wells where benzothiophene was absent. In a distance of 130 m 3 µg/L of benzothiophene and 5 µg/L of 3-methylbenzothiophene were still present.

The 3-ring heterocyclic compound dibenzofuran was determined in high concentrations up to 970 µg/L in the first line of wells. The compound represents the heterocyclic compound with the highest mass load (4 kg/a) at the Düsseldorf-Flingern field site. In comparison to its N-heterocyclic analog carbazole a higher distribution and level of concentration all over the site was present. It was still determined in some wells in a distance of 85 m from the source, where carbazole was no more present. Furthermore, in well 19071, 130 m from the source, dibenzofuran was found in 0.6 µg/L. In contrast, the S-analog dibenzothiophene was only determined in the first and second line of wells.

While for the most compounds investigated a clear reduction in concentration over the field site was observed, several methyl- and dimethylsubstituted benzofurans, dibenzofuran and benzothiophenes an increase in concentration within the flow direction was found.

Homocycles/PAHs. Acenaphthene was the compound present in highest concentrations within all compounds investigated. In the first line of wells a mass load of 10 kg/a enters the field in concentrations up to 1300 µg/L. In contrast to the other compounds investigated for acenaphthene a decrease with concentration within the depth was absent. Its importance was emphasised by the occurrence, even in high concentrations downgradient the plume and the fact that it was found in almost every sample analysed. Especially downgradient acenaphthene was determined in relevant concentrations, even in 130 m distance 78 µg/L were detected. Fluorene, the angular analog of acenaphthene, showed similar distribution. It entered the field site in a mass of 4 kg/a. However, when forming the ratio fluorene/acenaphthene a higher decrease of fluorene over the field site with the groundwater flow was indicated.

Within variations both compounds naphthalene and 1-methylnaphthalene were detected in a similar range of concentration in the first line of wells, with mass loads of 7 and 4 kg/a, respectively. When looking downgradient 1-methylnaphthalene was found more often and generally in higher concentrations than naphthalene. However, both compounds were still present in well 19071 with concentrations of 2 µg/L of naphthalene and 24 µg/L of 1-methylnaphthalene. In comparison 2-methylnaphthalene was determined in a small number of wells, and entered the field with a mass load of only 0.5 kg/a. Even in the first line of

wells, where naphthalene and 1-methylnaphthalene were found in relevant concentrations in every well and every depth, 2-methylnaphthalene was not detected frequently. Downstream only few samples contained 2-methylnaphthalene in detectable concentrations.

In the first line of wells indene was often found in higher concentrations than indan (1.1 and 0.5 kg/a, respectively). When looking downgradient this tendency changed. In the fourth line, indan was present in concentrations up to ~8 times higher compared to well 19209 and furthermore it was still found in 130 m distance. 1-Indanone was only detected in few wells in 19214 (line 1) and 19209 (line 4).

Hydroxylated compounds. 1-Naphthol and 2-naphthol were determined in several samples of line 1, while both were no more detectable downstream. In contrast, the hydroxylated analog of acenaphthene, 1-acenaphthenol, was found widespread over the site, while concentrations were generally about 1-2 µg/L in the first line as well as in line 4 downstream.

Distribution of compounds within the field site

In Figure 18 the distribution of quinoline and isoquinoline compounds within the first four lines of wells with 5 levels below top ground surface is shown. The distribution of contaminants was analysed to search for the fate of compounds in the plume with respect to correlations within the heterocycles.

As described above quinoline, isoquinoline and hydroxylated analogs showed high variances in concentration. The hydroxylated analogs were generally present in higher amounts than the non-substituted ones. All of these were mostly found on the left side of the test area in line 1 and 2, while also high concentrations were detected in the middle of line 4. Horizontal distribution of 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone clearly showed a decline of the maximum concentration from level 6.5 m to 8 m within the groundwater flow from line 1 to line 4. Interestingly, for both compounds a decrease in concentration from line 1 to line 3 is followed by a drastic increase in concentration to line 4. In contrast, quinoline was absent in line 4. The observations concerning isoquinoline and 1(2H)-isoquinolinone were similar to the data obtained for quinoline compounds in the horizontal as well as vertical distribution. 3,4-Dihydro-1(2H)-isoquinolinone was present only in very low concentrations over the whole field site. However, independent on the different levels of concentration distribution patterns of these compounds showed some similarities.

The observations of correlations within the mentioned quinoline compounds are enforced, when studying the distribution of other contaminants in the first line of wells, shown for selected compounds in Figure 19. Contaminants like benzofuran, 2-methylbenzofuran and benzothiophene showed major differences in compounds distribution. Especially in line 1, the highest contaminated one, maximum concentrations were found on the left as well as on the right side of the area.

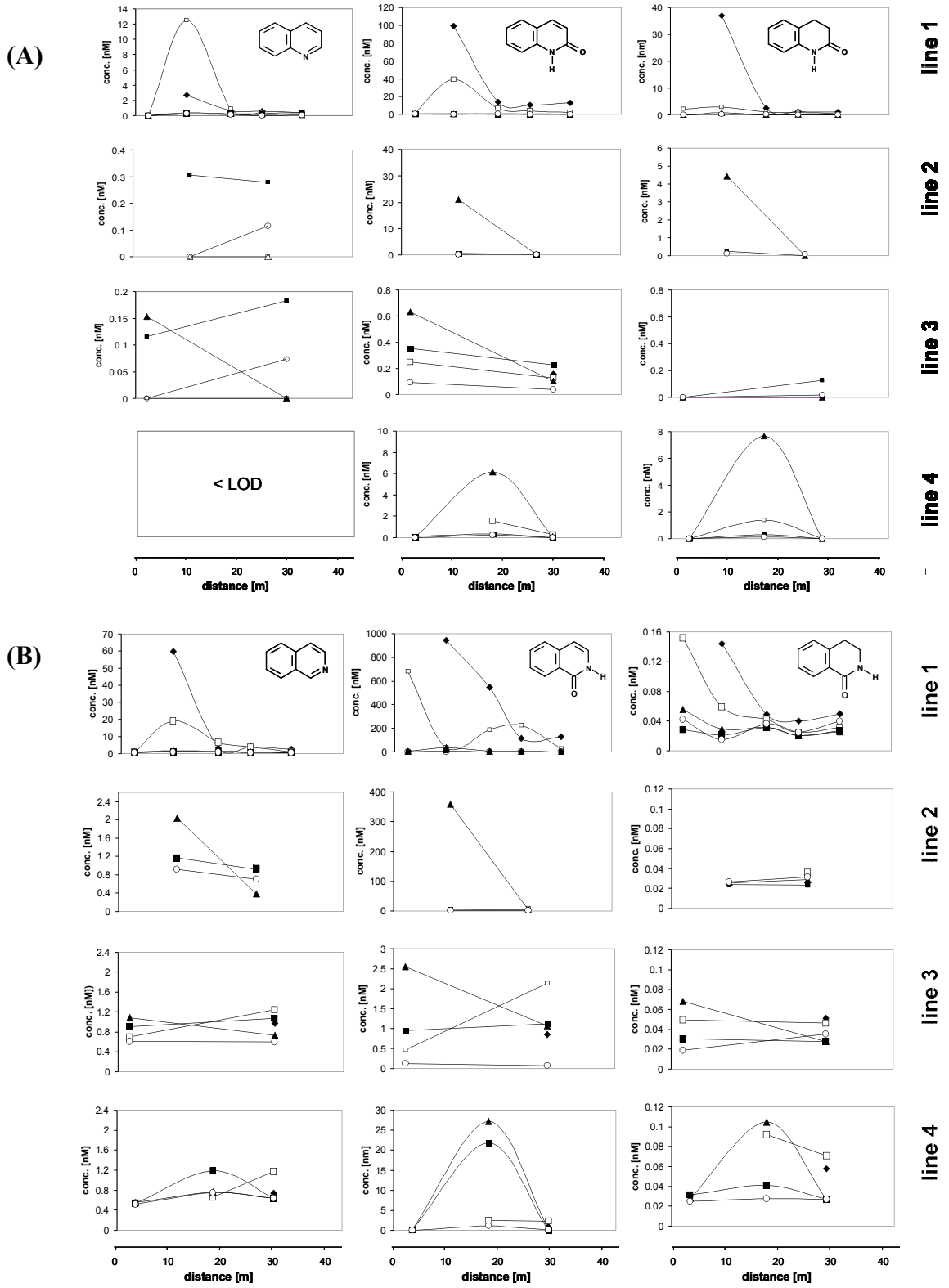


Figure 18: Distribution at the gasworks site Düsseldorf-Flingern of (A) quinoline, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone and (B) isoquinoline, 1(2H)-isoquinolinone, 3,4-dihydro-1(2H)-isoquinolinone. The data points represent the wells in 4 lines. The levels analysed are 6.5 m (◆), 7 m (□), 8 m (▲), 9 m (■), 10 m (○) below top ground surface.

Note differences in y-axis! For a better comparison of parent compounds and possible hydroxylated metabolites molar concentrations are given: quinoline and isoquinoline: 1 nM = 129 ng/L; 2(1H)-quinolinone and 1(2H)-isoquinolinone 1 nM = 145 ng/L; 3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone: 1 nM = 147 ng/L.

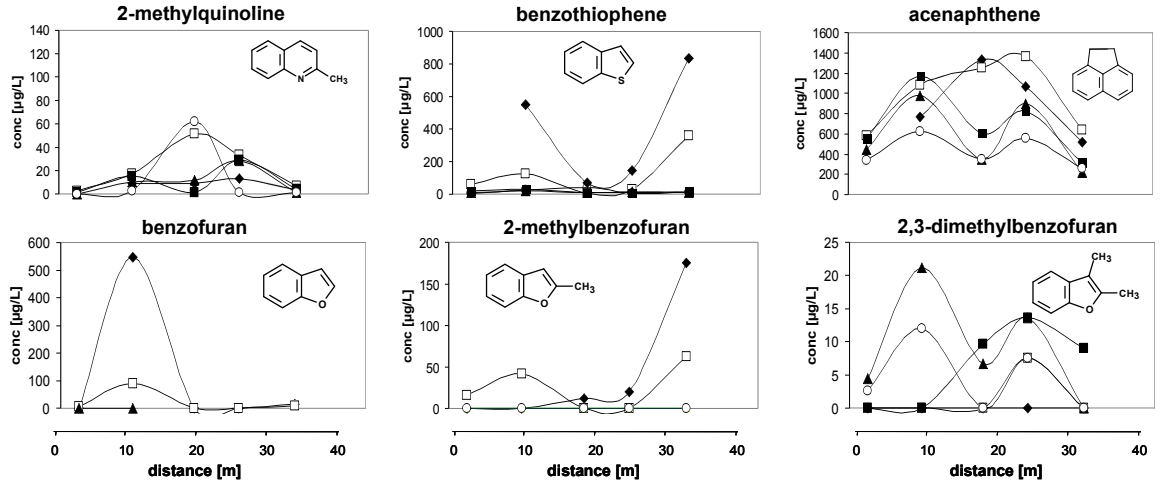


Figure 19: Distribution of selected heterocyclic compounds: 2-methylquinoline, benzothiophene, benzofuran, 2-methylbenzofuran and 2,3-dimethylbenzofuran as well as acenaphthene at the Düsseldorf-Flingern site. The data points show the wells in line 1. The levels analysed are 6.5 m (◆), 7 m (□), 8 m (▲), 9 m (■), 10 m (○) below top ground surface.

Comparison of different groups of compounds

A comparison of different groups of compounds was studied to determine the relevance of heterocyclic compounds in comparison to EPA-PAHs at the Düsseldorf-Flingern site. Data of EPA-PAHs were provided by Lars Richters; Stadtwerke Düsseldorf-Flingern.

The ratio of NSO-heterocycles in general to EPA-PAHs as well as the ratio of single groups of heterocycles to the EPA-PAHs determined in the five lines of wells downstream the source (0, 25, 50, 85 and 130 m) are shown in Figure 20 (A), while the ratios of heterocycles and acenaphthene are shown in Figure 20 (B).

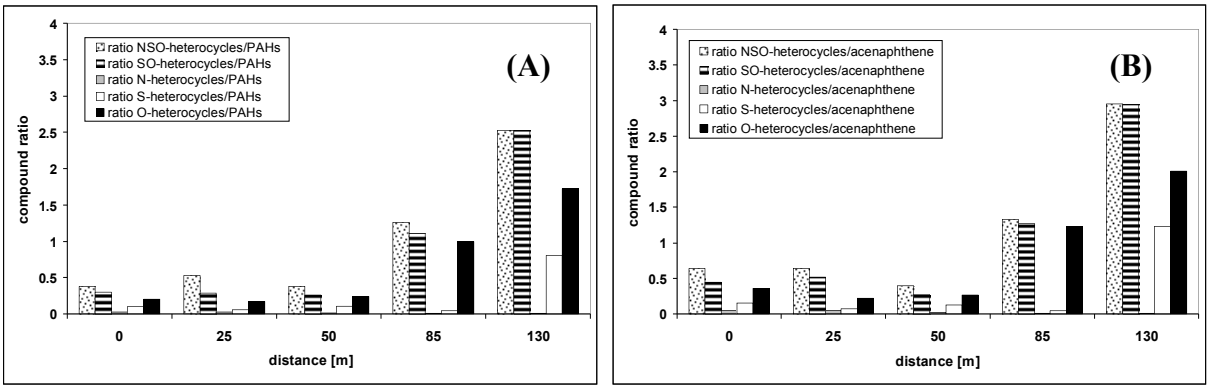


Figure 20: Ratio of NSO-heterocycles and single groups of heterocycles to (A) the group of EPA-PAHs as well as (B) acenaphthene at the Düsseldorf-Flingern site. The bars represent median values of concentrations of the lines of wells; the arrow marks the direction of groundwater flow.

The ratio of NSO-heterocycles/PAHs increases with distance from the source. This increase is most obvious in line 4, after a distance of 85 m.

A comparison of NSO-heterocycles/acenaphthene, the PAH found in highest concentration all over the field site, shows similar tendency. The ratio of heterocycles to acenaphthene is generally slightly higher than the ratios of heterocycles/EPA-PAHs as a result of high naphthalene concentrations, especially in the first lines.

The groups of NSO-heterocycles showed a ratio of N:S:O of **1:6:10** in line 1. In all lines within the field site, N-heterocycles play a minor role in the groundwater. In the lines of wells downstream, the N-heterocycles remain an insignificant group of compounds, while the ratios to S- and O-heterocycles vary (line 2: **1:4:5**; line 3: **1:5:7**; line 4: **1:0.4:2**; line 5: **1: 72:159**). In 130 m distance from the highest contaminated region the relevance of O- and S-heterocycles is clearly increased. N-Heterocycles were only detectable in minor amounts in this well (near their limit of detection with highly sensitive analytics). Generally, O-heterocycles were found of highest importance in every line of wells, followed by S-heterocycles, including their methylated and dimethylated analogs. The compounds detectable in a distance of 130 m are summarized in Table 8 (see page 55).

4.2.1.3 Wood impregnation site at Wülknitz

To get information about the spectrum and distribution of heterocycles present at the wood impregnation site at Wülknitz, 6 wells with up to 3 depths were analysed in July 2005. Groundwaters of two wells located near the contamination source and 4 wells situated downstream were studied (Figure 8). Large distances between the wells and changes in flow direction of the groundwater did not allow to perform confident mass balances as has been calculated for the Castrop-Rauxel as well as the Düsseldorf-Flingern site. However the decrease of compounds over the distance is compared to see indications for differences in compounds behaviour.

In Table 9 the concentrations in the highest contaminated well analysed, as well as decrease over the field site and remaining concentrations in well 14/01, the well most downstream, are summarized. The whole set of data is given additionally in Table 32 and 33.

N-Heterocycles. Quinoline was detected in wells near the source of contamination. In these wells 2(1H)-quinolinone as well as 3,4-dihydro-2(1H)-quinolinone were determined in obviously higher amounts. Even in the absence of quinoline, high amounts of 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone were present in well 24/05. Identical results occurred in well 14/01, up to ~250 m downstream the source: 2(1H)-quinolinone was still present in 0.02 µg/L, while concentrations of quinoline were below the limit of detection. 3,4-Dihydro-2(1H)-quinolinone was only detectable in samples where high concentrations of 2(1H)-quinolinone were determined. In well 20/05 (top) up to 68 µg/L of 3,4-dihydro-2(1H)-quinolinone was present which is 5 times the concentration of 2(1H)-quinolinone (Figure 51, appendix).

Isoquinoline was found in most groundwater samples, with the exception of well 06/98, which is located ~200 m from the highest contaminated well analysed. Isoquinoline concentration occurred in the same or higher range than concentration of quinoline. In general, more samples contained isoquinoline than quinoline (Figure 51, appendix).

Concentrations of 1(2H)-isoquinolinone were much higher than those of 2(1H)-quinolinone, for example in well 20/05 (top) 12 µg/L 2(1H)-quinolinone was found, while 2617 µg/L 1(2H)-isoquinolinone was present. 3,4-Dihydro-1(2H)-isoquinolinone was detected in all groundwater samples. While 3,4-dihydro-2(1H)-quinolinone concentrations decreased below limit of detection downstream, 3,4-dihydro-1(2H)-isoquinolinone was detectable even throughout the plume. In well 20/05 it reached a concentration of 189 µg/L.

Methylquinoline compounds were determined in well 20/05 in similar concentrations (up to 40 µg/L). However, no differences in decrease of the isomers over the field site were found. All methylquinolines were absent in well 14/01, the one most downstream. The same was true for 2,4- and 2,6-dimethylquinoline. These compounds were present in the highest contaminated well (20/05) in slightly higher concentrations than methylquinolines, while the decrease seemed to be similar over the field site. As has been described for quinoline and isoquinoline similar results were obtained for methylquinolines: methyl-2(1H)-quinolinones were found as an important group of compounds in the higher contaminated region, while the parent methylated quinoline compounds were present in lower concentrations. This is shown in Figure 52 for 4-methylquinoline and 4-methyl-2(1H)-quinolinone. Furthermore the sum of methylquinolines and the sum of methyl-2(1H)-quinolinones is presented.

Table 9: Concentration of compounds in the highest contaminated well (20/05), decrease over the field site (%) and compounds found in a distance of 100 and 250 m from the contamination source in well 13/01 and 14/01.

compound	14/01		13/01	20/05
	conc [µg/L]*	decrease [%]	decrease [%]	conc [µg/L]*
N-Heterocycles				
quinoline	<0.006	100	99.7	7.1
isoquinoline	<0.014	99.9	96.5	7.2
1-methylisoquinoline	<0.010	99.9	97.2	8.5
3-methylisoquinoline	0.006	99.9	96.8	5.6
2-methylquinoline	<0.006	100	97.6	10.9
4-methylquinoline	<0.004	100	94.3	26.1
6-methylquinoline	<0.004	100	96.8	7.2
2(1H)-quinolinone	0.014	99.9	94.9	25.4
1(2H)-isoquinolinone	0.110	100	94.8	1487
4(1H)-quinolinone	0.007	68.9	83.3	0.02
7-isoquinolinol	<0.004	100	100	6.8
3(2H)-isoquinolinone	0.009	99.8	99.1	4.1
3,4-dihydro-2(1H)-quinolinone	<0.008	100	97.7	27.5
3,4-dihydro-1(2H)-isoquinolinone	0.202	99.7	99.2	69.7
2-methyl-6-quinolinol	<0.004	100	99.4	9.5
4-methyl-2(1H)-quinolinone	0.065	99.7	94.5	552
6-methoxyquinoline	<0.003	100	96.2	2.9
1-methyl-2(1H)-quinolinone	0.032	99.4	95.3	10.2
2-methyl-4(1H)-quinolinone	0.111	99.5	89	40.8
2,4-dimethylquinoline	<0.006	100	99.1	25.3
2,6-dimethylquinoline	<0.006	100	99.1	20.1
1,2,3,4-tetrahydroquinoline	<0.013	99.8	98.2	2.5
7-hydroxy-4-methyl-2(1H)-quinolinone	0.012	99.9	99.7	9.8
carbazole	<0.4	100	+	16
6(5H)-phenanthridinone	<0.4	0**	+	<0.4
sum of N-Heterocycles	0.703	100	93.6	2382
S-Heterocycles				
benzothiophene	22	96.3	76.7	612
3-methylbenzothiophene	<0.8	99.6	58.3	97.9
methylbenzothiophene isomer	<0.8	99.6	99.6	99.3
sum of S-Heterocycles	22	97.2	77.4	809
O-Heterocycles				
benzofuran	<0.4	99.9	99.9	389
2-methylbenzofuran	19	93.6	39.4	300
2,3-dimethylbenzofuran	<0.4	99.8	75.3	94.8
dimethylbenzofuran isomer	1.0	98.3	60.1	57.2
dibenzofuran	<0.4	99.8	60.3	103
sum of O-Heterocycles	20	71.5	97.8	944
PAHs/Homocycles				
naphthalene	1.7	99.9	87.2	1143
2-naphthoic acid	<1.2	99.5	90.7	118
fluorene	<0.6	99.7	82.5	90.4
acenaphthene	3.6	99.2	98.7	423
sum of EPA-PAHs	5.0	99.5	82.4	1758
1-methylnaphthalene	2.7	99.4	91.2	458
2-methylnaphthalene	0.8	99.6	99.9	178
1,3-dimethylnaphthalene	<0.4	99.3	99.3	26.7
indene	<0.4	99.9	92.8	386
indan	32	90.0	44.3	318
1-indanone	<0.4	99.9	99.9	210
1-naphthol	<0.4	99.9	89.9	239
2-naphthol	<0.4	99.9	99.9	172

*Average concentrations of the different depths are given.

In those cases, where the analytics indicated the absence of a compound, half of the compounds detection limit was used for the calculation of mean concentration, which was used for the calculation of mass loads. For compounds present in concentration near the detection limit even in the inflow the values of decrease in the mass balance may represent a clear underestimation. These cases are marked by (**).

In general, all quinoline compounds investigated showed a high decrease in concentration in the downstream. Concentrations were below or near the limit of detection for all compounds in a distance of 250 m.

Carbazole, the only 3-ring N-heterocyclic compound present was only detectable in a some wells. No decrease over the distance of well 20/05 to 13/01 was present, while the compound was absent in 250 m downstream.

S- and O-Heterocycles. 2-Ring S- and O-heterocyclic compounds were found to be of high relevance at the Wülknitz site.

Benzofuran was detected only in wells near the source, while its methylated analogs were widely distributed. 2-Methylbenzofuran was detectable in all wells analysed and also 2,3-dimethylbenzofuran was found even in a distance of about 200 m. Furthermore, another isomeric dimethylated analog was present, which could not be identified clearly because of limited reference compounds. This isomer showed the same distribution behaviour as well as level of concentration as the 2-methylisomer. Benzofuran and 2-methylbenzofuran were determined in similar concentrations in the highest contaminated region (well 20/05). Over a distance of 100 m, the concentration of benzofuran decreased up to 100 %, while the decrease of 2-methylbenzofuran was only about 40 %. The decrease of 2,3-dimethylbenzofuran was notable, about 75 %. Concentrations up to 74 µg/L of 2-methylbenzofuran and 6 µg/L 2,3-dimethylbenzofuran were found, even in the two wells 06/98 and 14/01 downstream.

Dibenzofuran, the 3-ring O-heterocyclic analog, was present in lower concentration than the 2-ring O-heterocyclic compound benzofuran in the highest contaminated well. The decrease of the less water soluble dibenzofuran was only about 60 % of the initial concentration over a distance of 100 m. However, dibenzofuran was absent in any of the two wells downstream.

The S-heterocyclic compound benzothiophene was detectable all over the site. Up to 1047 µg/L were present in the highest contaminated well. A reduction of 77 % of the initial concentration found in well 20/05 was seen over a distance of 100 m. However, even 250 m downstream a concentration up to 32 µg/L (4 % of source concentration) was found. 3-Methylbenzothiophene was present in lower concentration in the highest contaminated well (225 µg/L). However, the decrease in concentration was only about 58 % over a distance of 100 m. In a distance of 200 m (well 06/98) 3-methylbenzothiophene was still detectable (19 µg/L), while in 250 m (well 14/01) it was absent. A second isomer of methylbenzothiophene showed a similar level of concentration near the source but higher decrease in concentration with the direction of the groundwater flow.

PAHs/Homocycles. Naphthalene was found as the main EPA-PAH in the highest contaminated wells (1143 µg/L), followed by acenaphthene (423 µg/L). Fluorene was present in lower concentration (90 µg/L). Although naphthalene was present in high concentration it was eliminated in higher amounts than acenaphthene within a distance of 250 m: the initial concentration of naphthalene decreased to 0.1 %, while 0.8 % of the initial acenaphthene concentration still remained. Fluorene was eliminated completely.

1-Methyl- and 2-methylnaphthalene were found in a similar range of concentration in most wells near the source, while the tendency of lower concentrations of 2-methylnaphthalene was indicated. In the wells downstream (well 13/01 and 6/98) only 1-methylnaphthalene was

detectable, but both isomers were found in well 14/01. However, 2-methylnaphthalene was present in a less number of wells than 1-methylnaphthalene.

Indan and indene were determined in the same range of concentration than 1-methyl- and 2-methylnaphthalene in well 20/05. The high importance of indan was indicated by its low decrease over a distance of 100 m, which was only about 44 % and only up to 90 % in 250 m distance. In contrast, indene decreased up to 93 % in 100 m and was completely absent in a distance of 250 m.

Hydroxylated compounds. Several hydroxylated compounds were found, especially in well 20/05, the well with highest level of contamination.

1- and 2-naphthol were also found in some other wells (24/05, 13/01, 21/05). 2-Naphthoic acid was present in well 24/05, 13/01 and 20/05 up to 190 µg/L. 1-Indanone was only found in the highest contaminated region (well 20/05).

The 3-ring N-heterocyclic compound 5(6H)-phenanthridinone was determined even in a distance of ~100 m (well 13/01) of the source as well as on the frame of the source (well 24/05 and 21/05), while it was absent in the highest contaminated well 20/05.

In general, all these hydroxylated compounds were not detectable in a distance of 250 m.

Comparison of different groups of compounds

In the highest contaminated well 20/05 the concentration of the sum of NSO-heterocyclic compounds was about a factor 4 higher than the concentration of the sum of 16 EPA-PAHs (data kindly provided by S. Schönekerl, university of Dresden and the engineer office Vogtland, Oelsnitz) (Figure 21 A). In about 250 m distance from the source the group of NSO-heterocyclic compounds increased in comparison to EPA-PAHs and reached concentrations 6 times the EPA-PAHs concentration.

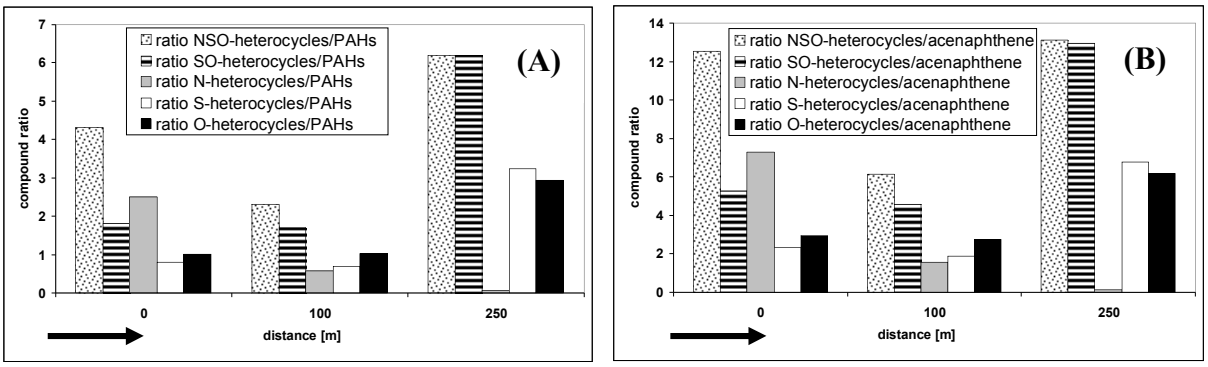


Figure 21: Ratio of NSO-heterocycles and single groups of heterocycles to (A) the group of EPA-PAHs as well as (B) acenaphthene at the Wülknitz site. The bars represent median values of concentrations of the lines of wells; the arrow marks the direction of groundwater flow.

More than half of the sum of NSO-heterocyclic concentration is represented by the N-heterocyclic compounds in well 20/05. The ratio of N:S:O-heterocyclic compounds is about **1:0.3:0.4**. Over a distance of ~100 m (between well 20/05 and 13/01) a decrease in concentration of 85 % was detected for the sum of NSO-heterocycles, but a lower decrease was found for the sum of SO-heterocyclic compounds (74 %), leading to a change of the ratio of N:S:O to **1:1.2:1.8**. The decrease of PAHs was about 82 %. The main part of

decrease of NSO-heterocycles is due to the high reduction of N-heterocycles. N-heterocycles, which form 50 % of the sum of NSO-heterocycles in well 20/05 represented only 25 % in 100 m distance. This observation is strengthened with distance of ~250 m from the source. In well 14/01 the group of N-heterocycles was only 1.6 % of the sum of NSO-heterocycles and the N:S:O-ratio changed to **1:45:41**. In general, up to 2 % of the initial SO-heterocyclic concentration was found in a distance of 300 m, while 0.5 % of initial EPA-PAHs concentration was determined.

The comparison of heterocycles with acenaphthene -known as highly persistent PAH-compound- (Figure 21 B) shows even slightly higher importance of S- as well as O-heterocycles with the groundwater flow direction. The ratio of the group of S-heterocycles and O-heterocycles versus acenaphthene increased in 250 m downstream.

4.2.2 Site-directed analyses: Indications for microbial degradative potential

A comparison of distribution and level of concentration of contaminants at the field sites as described in the previous chapter may give some first hints for biodegradation at the field sites, which is the main factor influencing distribution and concentration of compounds with similar physicochemical characteristics.

However, to understand the processes of natural attenuation it is of high importance to support these findings with knowledge from degradation studies. Generally, studies about the anaerobic degradation of heterocycles are scarce. There exist few studies dealing with the presence of only one compound, while the degradation of complex mixtures as normally present in the environment is rarely analysed.

Therefore, the following approaches were done:

1. Microcosms were performed using groundwater with the authentic contamination (from the Düsseldorf-Flingern and Wülknitz site). The influence of variation of redox conditions as well as addition of reducing agents was studied.
2. The anaerobic microbial activity in an on-site column filled with aquifer material and contaminated groundwater from the Wülknitz field site was studied.
3. Contaminants plumes at the Düsseldorf-Flingern site using a high-resolution well, which allowed the analyses of samples with a vertical resolution within 3 cm, were compared with each other as well as the plume of electron acceptors.

4.2.2.1 Microbial degradation in microcosms

Anaerobic microcosms with groundwater from the Düsseldorf-Flingern site

Groundwater of well 19201 (7 m depth) was investigated for its microbial degradative activity towards heterocycles and selected homocycles under sulfate-, iron- and nitrate-reducing redox conditions. Well 19201 is located near the source of the plume as shown in Figure 6.

In general, an incubation period of 2.5 years resulted in an elimination of only few compounds of the spectrum of pollutants analysed. Considerable differences in microbial activity were seen with the microbial population under the different redox conditions tested. Highest rates of degradation were found under sulfate-reducing, followed by iron-reducing conditions. Only few compounds show a reduction in concentration and only to a lower extent, when nitrate was given as electron acceptor (Table 10).

Within the sulfate-reducing microcosms the microbial activity differs with respect of the reducing agent added. Overall, the extent of transformed compounds was comparable in the sulfate-reducing microcosms. But the addition of Ti-NTA led to higher transformation activity than the supply of Na-dithionite. Moench and Zeikus (1983) reported that nitrilotriacetic acid is unknown to be used as carbon source. A strong influence of reducing agent on transformation rate (under sulfate-reducing and methanogenic conditions) of quinoline and indole was found by Licht et al. (1997b), where the presence of dithionite resulted in slower transformation of indole or quinoline compared to the titanium(III)citrate assay.

The concentration of a huge number of quinoline compounds, naphthalene and methylnaphthalenes decreased considerably. Inspection on the different quinoline compounds showed profound degradation of the non-substituted parent compounds quinoline and isoquinoline. The same was true for the methylated analogs 4-methyl- and

6-methylquinoline as well as 3-methylisoquinoline, while transformation of 2-methylquinoline was absent and 1-methylisoquinoline was transformed less. When comparing the parent compounds with the hydroxylated compounds, 2(1H)-quinolinone, 3,4 dihydro-2(1H)-quinolinone and 1(2H)-isoquinolinone, the hydroxylated compounds were found to be less degradable. Independent of reducing agent used 1(2H)-isoquinolinone was persistent over the whole incubation period, while concentrations of 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone decreased in Ti-NTA-containing microcosms up to 100 %. The same result was obtained for 4-methyl-2(1H)-quinolinone, which only disappeared in the Ti-NTA-containing microcosms.

Out of these compounds the transformation of further NSO-heterocyclic compounds was absent with the exception of benzothiophene, which minor decreased in minor concentrations.

In the iron-reducing microcosms a mix of iron- as well as sulfate-reducing processes were present, as has been shown by the formation of dissolved iron as well as a decrease in concentration of sulfate. In addition to quinoline compounds only few compounds were degraded. These were benzothiophene, benzofuran, naphthalene, 1- as well as 2-methylnaphthalene. In contrast to sulfate-reducing conditions transformation of carbazole was found in the complete set of iron-reducing microcosms. The addition of ascorbic acid into the microcosms led to a higher degree of transformation for some compounds such as 1(2H)-isoquinolinone, 2-methylquinoline, 1-methylisoquinoline, and 2,4-dimethylquinoline. In contrast, an inhibition of transformation by the presence of ascorbic acid was observed for quinoline, 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone, naphthalene, 1-methyl- and 2-methylnaphthalene. Furthermore, anthraquinone-2,6-disulphonic acid (AQDS) was added as electron shuttle with the aim to extent the availability of $Fe(OH)_3$ as electron acceptor. A catalytic role of this shuttle is known. If the bacteria oxidize the organic compound, the electron shuttle is reduced. The shuttle then passes the electron onto $Fe(OH)_3$ and re-oxidizes itself (Lovley et al., 1996, 1999). However, the addition of AQDS did not clearly effect the degradative potential in microcosms tested here.

Differences in the microbial activity were obvious in the denitrifying microcosms when analysing the amount of nitrate reduced. The minor formation of nitrite indicated insignificantusage of nitrate as electron acceptor. The addition of ascorbic acid led to little higher microbial activity; however, only a small number of compounds were degraded. 2(1H)-Quinolinone and isoquinoline were the only quinoline compounds whose concentration decreased up to 32-36 % in maximum. Some elimination of benzothiophene, 2-methylbenzofuran and carbazole was found in the denitrifying microcosms. Furthermore, within the homocyclic compounds investigated naphthalene and 1-methylnaphthalene as well as indan represented the minority of compounds showing a decrease in concentration.

Table 10: Results from anaerobic microcosm experiments with groundwater of well 19201, 7 m depth of the Düsseldorf-Flingern site; decrease in concentration of organic compound, used amount of sulfate and nitrate, formed amount of iron(II) and nitrite are given.

compound	concen-tration at the beginning* [µg/L]	sulfate-reducing conditions		iron-reducing conditions				nitrate-reducing conditions	
		1	2	3	4	5	6	7	8
N-Heterocycles									
quinoline	0.33	++	++	++	++	-	°	++	-
isoquinoline	4.7	++	++	++	(+)	++	++	+	(+)
1-methylisoquinoline	2.6	-	(+)	+	-	++	+	-	-
3-methylisoquinoline	0.91	+	++	++	+	+	(+)	-	-
2-methylquinoline	61	-	-	-	-	+	(+)	-	-
4-methylquinoline	0.54	++	++	++	++	++	++	-	-
6-methylquinoline	1.0	+	++	++	+	+	+	-	-
Σ methylquinoline	15	-	(+)	+	(+)	(+)	(+)	-	-
2(1H)-quinolinone	7.4	-	++	-	(+)	-	-	-	(+)
1(2H)-isoquinolinone	25	-	-	-	-	+	(+)	-	-
3,4-dihydro-2(1H)-quinolinone	0.51	-	++	-	(+)	-	-	-	-
2-methyl-6-quinolinol	0.02	-	-	-	-	-	-	-	-
4-methyl-2(1H)-quinolinone	11	-	++	-	-	-	-	-	-
1-methyl-2(1H)-quinolinone	0.09	-	-	-	-	-	-	-	-
2-methyl-4(1H)-quinolinone	0.52	-	-	+	(+)	+	(+)	-	-
Σ methyl-2(1H)-quinolinone	52	-	(+)	-	-	-	-	-	-
2,4-dimethylquinoline	47	-	-	-	-	+	+	-	-
2,6-dimethylquinoline	1.8	-	-	+	(+)	+	+	-	-
Σ dimethylquinolines	58	-	-	(+)	-	+	+	-	-
carbazole	66	-	-	+	(+)	(+)	(+)	-	+
6(5H)-phenanthridinone	23	-	-	-	-	-	-	-	-
S-Heterocycles									
benzothiophene	56	-	(+)	+	+	-	+	-	(+)
3-methylbenzothiophene	62	-	-	-	-	-	-	-	-
O-Heterocycles									
benzofuran	51	-	-	(+)	-	-	-	-	-
2-methylbenzofuran	3.2	-	-	-	-	-	-	-	++
dibenzofuran	144	-	-	-	-	-	-	-	-
Homocycles/PAHs									
naphthalene	290	(+)	++	++	++	-	+	-	(+)
1-methylnaphthalene	346	(+)	++	(+)	(+)	-	(+)	-	(+)
2-methylnaphthalene	9.8	++	++	++	++	-	++	-	-
indan	62	-	-	-	-	-	(+)	-	(+)
acenaphthene	220	-	-	-	-	-	-	-	-
fluorene	46	-	-	-	-	-	-	-	-
Electron acceptors									
decrease of sulfate (mg/L)	/	-	118	8	19	92	93	-	-
formation of iron (II) (mg/L)	/	n.d.	n.d.	1967	1781	8158	8518	n.d.	n.d.
decrease of nitrate (mg/L)	/	n.d.	n.d.	<1	<1	<1	<1	5	250
formation of nitrite (mg/L)	/	-	-	-	-	-	-	4	101

Legend: ++ = 80-100 % decrease; + = 40-80 % decrease; (+) = 10-40 % decrease, - = <10 % decrease; ° no result; decrease in controls; * mean of all parallels and different redox conditions was formed.

1 = sulfate-reducing, addition of Na-dithionite, 2 = sulfate-reducing, addition of Ti-NTA, 3 = iron-reducing conditions, 4 = iron-reducing conditions, addition of AQDS, 5 = iron-reducing conditions, addition of ascorbic acid, 6 = iron-reducing conditions, addition of AQDS and ascorbic acid, 7 = nitrate-reducing conditions, 8 = nitrate-reducing conditions, addition of ascorbic acid.

n.d. = not determined; the sum of methylquinolines includes all methylquinolines which show a transition of 144/115. The sum of methyl-2(1H)-quinolinones includes the transition of 160/115. Therefore, the sums are only minor influenced by 2-methylquinoline and 1-methylisoquinoline.

Anaerobic microcosms with groundwater from the Wülknitz site

The degradative potential of groundwater of well IW1, IW2, IW5, 13/01 as well as 1/97 (Figure 8 for the location of wells) was studied in microcosms. The wells were chosen to represent a good distribution all over the field site. In one assay of microcosms groundwater samples were studied directly without any addition, while in the other one amorphous iron hydroxide was added as electron acceptor. Results were obtained after 1 and 2 years of incubation in correlation to inactivated control microcosms. After 2 years the group of quinoline compounds was additionally studied in more detail. In the following paragraph results obtained after 2 years of incubation are presented.

The data showed a different extend of the transformation potential, depending on groundwater samples investigated. Decrease in concentration of a high number of compounds in different groundwater samples is shown in Figure 23. In general the degradative activity in batches without any additional electron acceptor were similar to those where iron hydroxide was added. When looking for the electron acceptors (SO_4^{2-}, NO_3^-, Fe^{3+}) several processes might occur in the microcosms. A clear correlation of decrease of compounds and redox conditions is not possible. Reduction of nitrate was found of high relevance in all batches. The batches without any addition are a combination of nitrate- and sulfate-reducing conditions, and those with additional iron, are a combination of nitrate-, iron- and sometimes sulfate-reducing conditions (for decrease of electron acceptors in the microcosms see Table 35).

Well IW1 is located near the main source of contamination beneath the destroyed hall of impregnation. It is the groundwater sample used for microcosms containing the highest level of contamination. The groundwater is contaminated with PAHs up to 7 mg/L, quinolines up to 18 mg/L and other heterocycles up to 4 mg/L. After an incubation time of 1 year nitrate- and sulfate-reduction as well as Fe(II)-formation was observed, however none of the analysed compounds was degraded. Only the quinoline concentration decreased, while the concentration of 2(1H)-quinolinone increased.

One year later, after a total incubation time of two years, decrease of several PAHs, quinolines and other heterocycles was determined. Compounds like quinoline, isoquinoline, naphthalene and 2-methylnaphthalene were eliminated at highest rate under sulfate- as well as iron-reducing conditions, generally in the range of 80 %. A degradation of 3-ring compounds carbazole, 6(5H)-phenanthridinone, dibenzofuran and fluorene was present in both assays. The microbial activity was obviously higher when iron hydroxide was added.

Decrease in concentration was found for all methyl(iso)quinolines investigated. However, methylquinolines substituted in position 2 (position 1 for methylisoquinoline) were decreased less. Furthermore, the degradation of the O-heterocyclic compound benzofuran and its S-analog benzothiophene occurred. Both were transformed in amounts between 25-60 %, with a slightly higher transformation of benzothiophene. Comparing benzofuran with its methylated analogs, a tendency of highest transformation of benzofuran, followed by 2-methylbenzofuran was observed. For 2,3-dimethylbenzofuran only minor transformation was found. Differences between the degradative potential were obvious for 1-methyl- and 2-methylnaphthalene. While 2-methylnaphthalene decreased at about 90-100 %, 1-methyl-naphthalene was more resistant and an elimination of only 20 % took place.

Concentrations of 2(1H)-quinolinone, 2-naphthol and 1-indanone after two years, higher than at the beginning showed the formation of these compounds in the meantime. The low decrease (10-20 %) of hydroxylated compounds 1(2H)-isoquinolinone, 3,4-dihydro-2(1H)-quinolinone, 4-methyl-2(1H)-quinolinone may be due to the formation during the incubation time.

Acenaphthene, 1,3-dimethylnaphthalene, indan and indene were the homocyclic compounds investigated which were worst transformed in both types of microcosms. Generally the results of degradability showed that even at high levels of concentration, where toxic effects may be expected, microbial activity is present.

Well IW2 is located in the northern part of the main source at the Wülknitz site. The concentration of aromatic compounds is about 70-80 % less than in well IW1. An important part of the aromatic compounds is represented by 5 mg/L of quinolines, followed by 1.3 mg/L of EPA-PAHs and 0.8 mg/L of other heterocyclic compounds. The main compounds present at the beginning of the experiment were 2(1H)-quinolinone (3.4 mg/L), 1(2H)-isoquinolinone (1.2 mg/L) and naphthalene (0.7 mg/L).

Decrease of hydroxylated quinolines, 1(2H)-isoquinolinone and 2(1H)-quinolinone, started after one year when iron hydroxide was added, while only 2(1H)-quinolinone decreased in microcosms without any addition. These results were improved after 2 years, where 1(2H)-isoquinolinone was no more detectable in microcosms with iron, while it remained in the groundwater without iron hydroxide addition.

3,4-Dihydro-2(1H)-quinolinone, 2-methyl-4(1H)-quinolinone, 4-methyl-2(1H)-quinolinone were the quinoline compounds which decreased at high degree. Different substituted methyl- and dimethylquinolines showed in general low decreasing concentration up to ~20 %.

For the other heterocyclic compounds and PAHs higher decrease in concentration was found without iron hydroxide addition. Also for acenaphthene up to ~60 % decrease was reached. The tendency of a better degradability of benzofuran in contrast to its methylsubstituted analogs was observed when iron hydroxide was absent.

Groundwater samples from well IW5 (downstream the source ~160 m) and 1/97 (data not shown) showed highest biodegradative activity. In groundwater of both wells, independent on the redox-conditions, all investigated compounds were decreased to a level near or under the limit of detection. Starting levels of concentration of these groundwater samples were in a similar range, while the composition of group of compounds was different: concentration of PAHs in well 1/97 were about 2.3 mg/L, while they were lower in well IW5 (0.4 mg/L). In contrast, quinoline concentrations in well 1/97 were lower (0.2 mg/L) than in IW5 (0.8 mg/L). Amounts of other heterocyclic compounds were comparable. Therefore, the high degradative activity was not focused on special groups of compounds.

While well IW5 and 13/01 (~190 m downstream the source) were only separated by a distance of ~30 m high differences in degradative activity were found. Concentrations of PAHs and other heterocyclic compounds were almost identical in IW5 and 13/01 (300 mg/L and 800 mg/L, respectively), while quinoline compounds were present in a much lower concentration (0.02 mg/L) in 13/01.

A significant decrease in concentration started after one year of incubation in microcosms with groundwater from well 13/01. However, with the exception of most quinoline compounds present, only a slight decrease in concentration was found for other heterocyclic compounds as well as PAHs investigated.

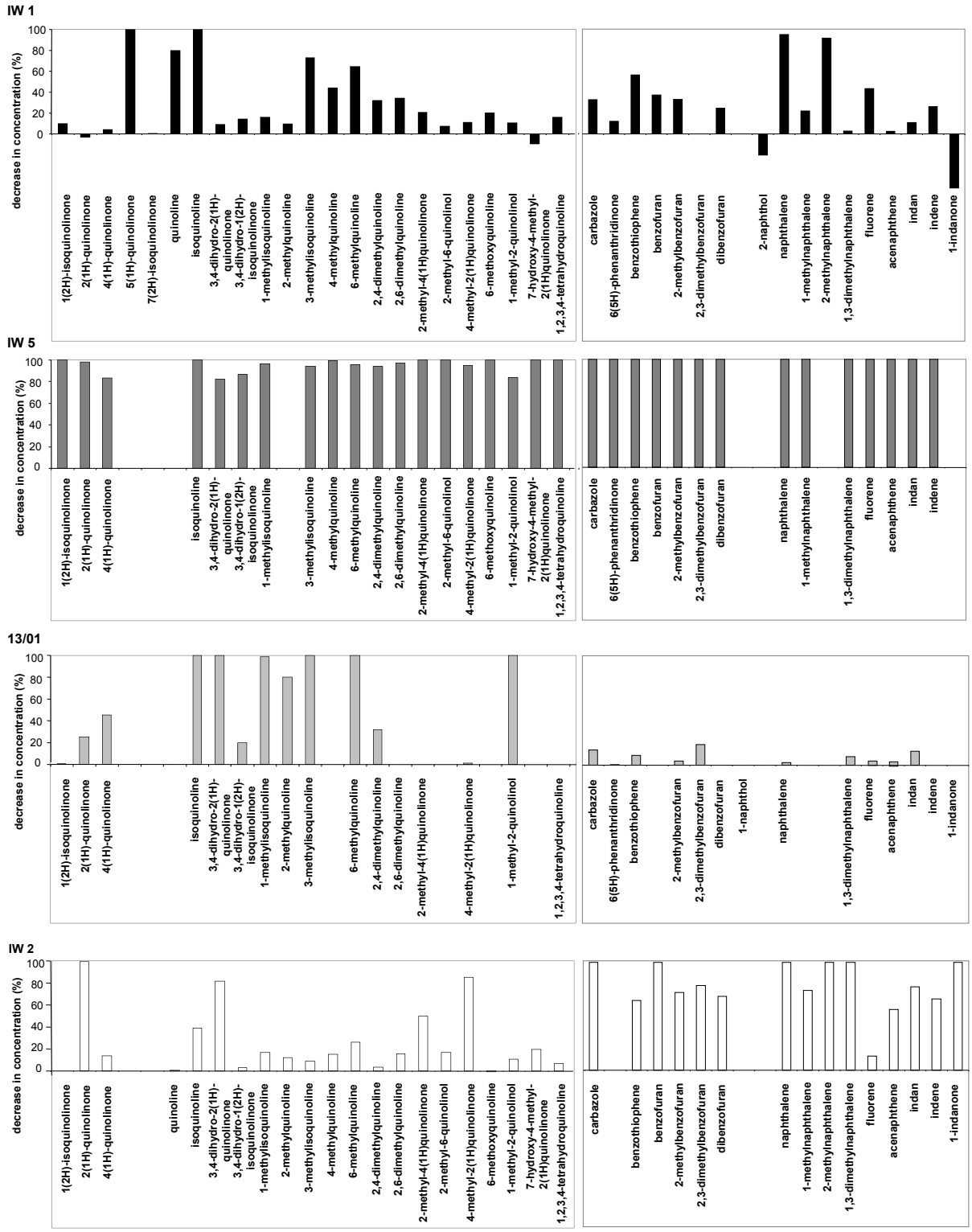


Figure 22: Decrease in concentration (%) of several tar oil components in microcosms with groundwater under sulfate-reducing conditions (addition of ascorbic acid) over a time period of 2 years. Decrease is shown in relation to inactivated microcosms as controls. Recalcitrance of a chemical is shown by the absence of any bar, but given name of the chemical.

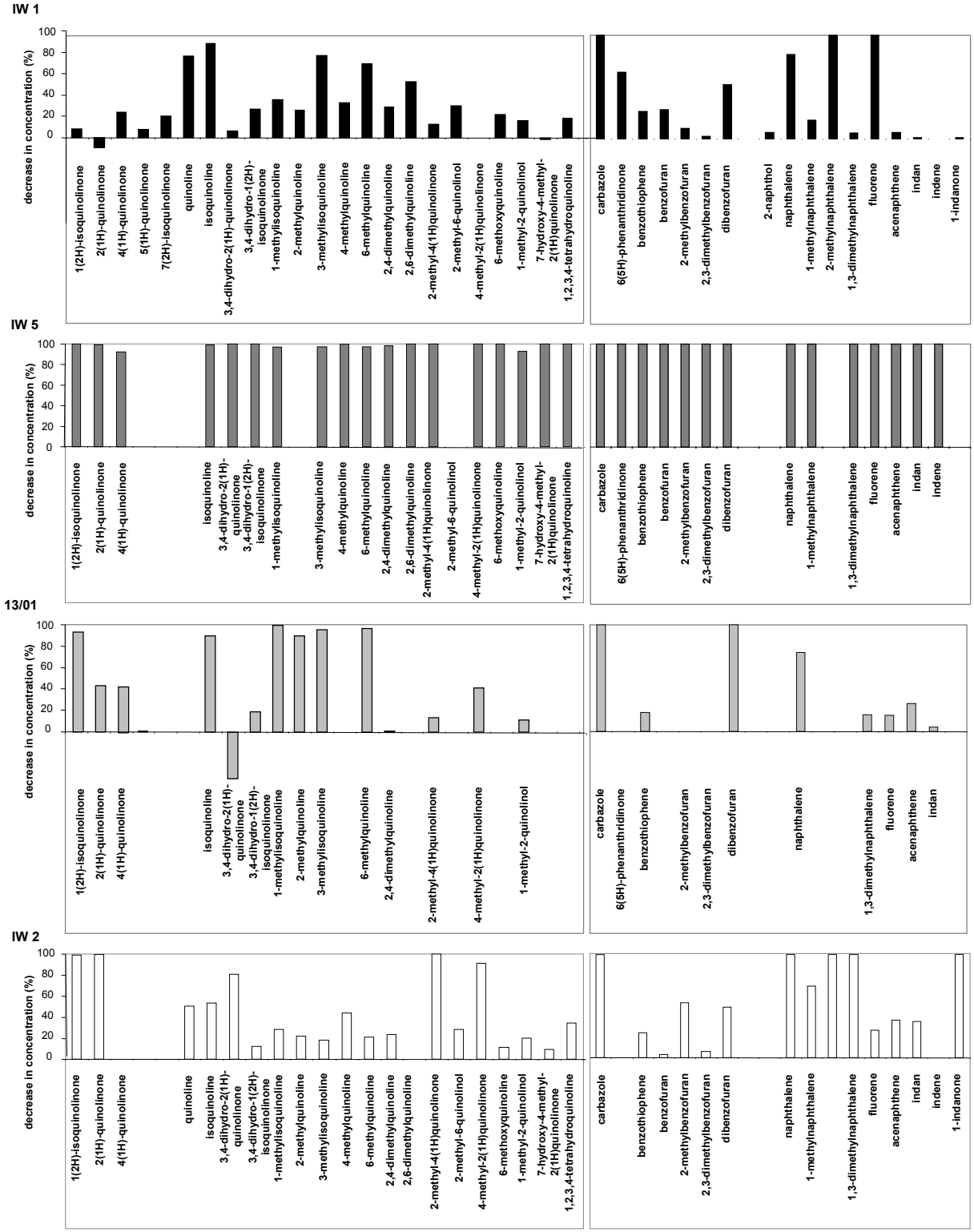


Figure 23: Decrease in concentration (%) of several tar oil components in microcosms with groundwater under iron-reducing conditions (addition of iron hydroxide and ascorbic acid) over a time period of 2 years. Decrease is shown in relation to inactivated microcosms as controls. Recalcitrance of a chemical is shown by the absence of any bar, but given name of the chemical.

4.2.2.2 Microbial degradation in on-site columns

The aim of this part of the study was to find indications for microbial degradation of NSO-heterocyclic compounds as well as PAHs under conditions, which simulate field conditions. Therefore, column experiments with aquifer material and groundwater of the Wülknitz site were performed to obtain conclusions from the field site. Samples were taken over a time period of almost one year, with at least 10 samplings.

In general, microbial degradation in the columns was clearly indicated by the comparison of concentrations of the active columns with data from the abiotic controls. When looking at DOC-data a decrease of organic compounds was determined at every sampling time in the biotic columns (data not shown). The extension of the intervals from 4 to 6 weeks showed increasing degradation. The redox potential was found to be within -143 to -387 mV in the columns, with lower values in the active columns. A decrease in concentration of sulfate was not observed (even if degradation of all compounds present in the groundwater occurs completely, resulting in CO_2 + H_2O, the amount of sulfate reduced was in the one-digit mg/L-range and therefore within standard deviations of the analyses). However, an increase in concentrations of Fe(II) (2-3 mg/L) was found indicating the presence of iron-reducing conditions. In Table 11 the percentage in decrease of concentration due to microbial degradation of different heterocyclic as well as homocyclic compounds is shown. Percentages are the result of averaging data from the 10 samplings. The decrease in concentration within the experimental time is presented for selected compounds in Figure 24.

Comparing the relative decrease in concentration in columns filled with groundwater from different depths (MP = 9-13 m and UP = 16-20 m), similar results - favour of the identical compounds, minor or no degradation of others - were indicated.

N-Heterocycles. For most quinoline compounds within a period of 4-6 weeks, 80-100 % decrease in concentration was found in the two active columns. Quinoline and isoquinoline were present in low but similar concentrations in the groundwater (2-4 µg/L). Quinoline was transformed up to 100 %, while isoquinoline showed slightly lower decrease (95-98 %). 2(1H)-Quinolinone and 1(2H)-isoquinolinone were present in concentrations up to a factor 10 higher. Both were transformed up to 86-99 %. The same was true for 3,4-dihydro-2(1H)-quinolinone. Only minor amounts of 3,4-dihydro-1(2H)-isoquinolinone were present while a clear decrease was absent.

4-Methylquinoline was the methylsubstituted quinoline (decrease 94-95 %) which was transformed best, followed by 6-methylquinoline and 3-methylisoquinoline (77-82 %) with comparable starting concentrations. Even for 2-methylquinoline and 1-methylisoquinoline a decrease was shown. The decrease was found in the range of 31-50 %, while the concentrations of these two compounds were about 10-fold higher than concentrations of the other isomers. For 2,4- and 2,6-dimethylquinoline no decrease was detected, while standard deviations were generally very high. The decrease of the 3-ring analog carbazole was found up to 20 %.

S-Heterocycles. Benzothiophene was the only S-heterocyclic compound identified. A decrease up to 14 % was detected.

O-Heterocycles. No decrease of 2-methylbenzofuran and 2,3-dimethylbenzofuran was determined. Variations of concentrations were relatively high and a clear trend could not be

observed. A decrease of the 3-ring-heterocyclic compound dibenzofuran was found up to 27 %.

Homocycles/PAHs. When looking for naphthalene, 1-methyl- as well as 2-methylnaphthalene a different degree in degradative activity was obvious. From the spectrum of compounds analysed, naphthalene was the compound present in highest amounts in the groundwater, but it was anyhow degraded up to 86 %. Comparing the degradative potential of the two methylated analogs it was clearly indicated that 2-methylnaphthalene was degraded in higher amounts (91-100 %) than 1-methylnaphthalene (only 11-30 %). Fluorene was transformed in a similar range than 1-methylnaphthalene, while indan and acenaphthene were decreased only in minor amounts.

Table 11: Decrease of hetero-and homocyclic compounds in the on-site columns of the Wülknitz-site.

compound	MP		UP	
	decrease [µg/L]	decrease [%]	decrease [µg/L]	decrease [%]
N-Heterocycles				
quinoline	2.1 ± 0.4	100 ± 0	3.8 ± 1.0	100 ± 0
isoquinoline	1.6 ± 0.8	95 ± 5	3.4 ± 1.3	98 ± 2
1-methylisoquinoline	0.6 ± 0.4	31± 25	0.5 ± 0.1	47 ± 10
3-methylisoquinoline	1.3 ± 0.4	77 ± 13	0.9 ± 0.1	81 ± 7
2-methylquinoline	28 ± 15	35 ± 18	12 ± 3	50 ± 7
4-methylquinoline	1.5 ± 0.8	95 ± 4	1.6 ± 0.5	94 ± 3
6-methylquinoline	1.1 ± 0.5	79 ± 18	0.8 ± 0.3	83 ± 11
Σ methylquinoline	7.4 ± 3.6	44 ± 14	5.1 ± 0.7	62 ± 4
2(1H)-quinolinone	16 ± 8	86 ± 2	39 ± 16	97 ± 1
1(2H)-isoquinolinone	20 ± 7	99 ± 0.3	14 ± 7	99 ± 0
4(1H)-quinolinone	0.09 ± 0.04	83 ± 11	0.10 ± 0.04	84 ± 19
3,4-dihydro-2(1H)-quinolinone	3.6 ± 1.6	85 ± 2	3.9 ±1.5	92 ± 2
3,4-dihydro-1(2H)-isoquinolinone	0.01 ± 0.02	7± 14	0.00 ± 0.02	-2 ± 10
4-methyl-2(1H)-quinolinone	7.4 ± 2.9	99 ± 1	3.7 ± 1.7	99 ± 1
2-methyl-4(1H)-quinolinone	0.06 ± 0.9	1 ± 15	-3.0 ± 5.9	-35 ± 75
Σ methyl-2(1H)-quinolinone	75 ± 18	22 ± 4	35 ± 11	20 ± 7
2,4-dimethylquinoline	-0.3 ± 0.5	-99 ± 203	0.0 ± 0.3	-36 ± 118
2,6-dimethylquinoline	0.12 ± 0.23	-5 ± 101	0.1 ± 0.2	22 ± 46
7-hydroxy-4-methyl-2(1H)-quinolinone	-0.17 ± 0.09	-108 ± 43	-0.13 ± 0.07	-181 ± 92
Σ dimethylquinolines	1.3 ± 2.8	4 ± 44	1.9 ± 1.0	37 ± 21
carbazole	13 ± 11	20 ± 16	8.3 ± 4.2	16 ± 10
S-Heterocycles				
benzothiophene	27 ± 11	14 ± 5	17 ± 14	11 ± 10
O-Heterocycles				
2-methylbenzofuran	9.4 ± 13.1	8 ± 13	1.9 ± 5.2	2 ± 7
2,3-dimethylbenzofuran	-2.9 ± 7.2	-23 ± 69	-3.5 ± 11.6	-0 ± 2
dibenzofuran	7.1 ± 5.5	8 ± 6	14 ± 6	27 ± 11
Homocycles/PAHs				
naphthalene	83 ± 14	86 ± 4	67 ± 9	85 ± 3
1-methylnaphthalene	7.4 ± 4.1	30 ± 19	2.2 ± 2.7	12 ± 17
2-methylnaphthalene	15 ± 9	100 ± 0	11 ± 9	91 ± 28
indan	3.2 ± 16	2 ± 8	4.1 ± 13.9	2 ± 10
indene	11 ± 11	50 ± 52	14 ± 9	71 ± 43
fluorene	16 ± 6	44 ± 16	8.4 ± 7.9	31 ± 18
acenaphthene	12 ± 16	5 ± 6	22 ± 14	10 ± 6

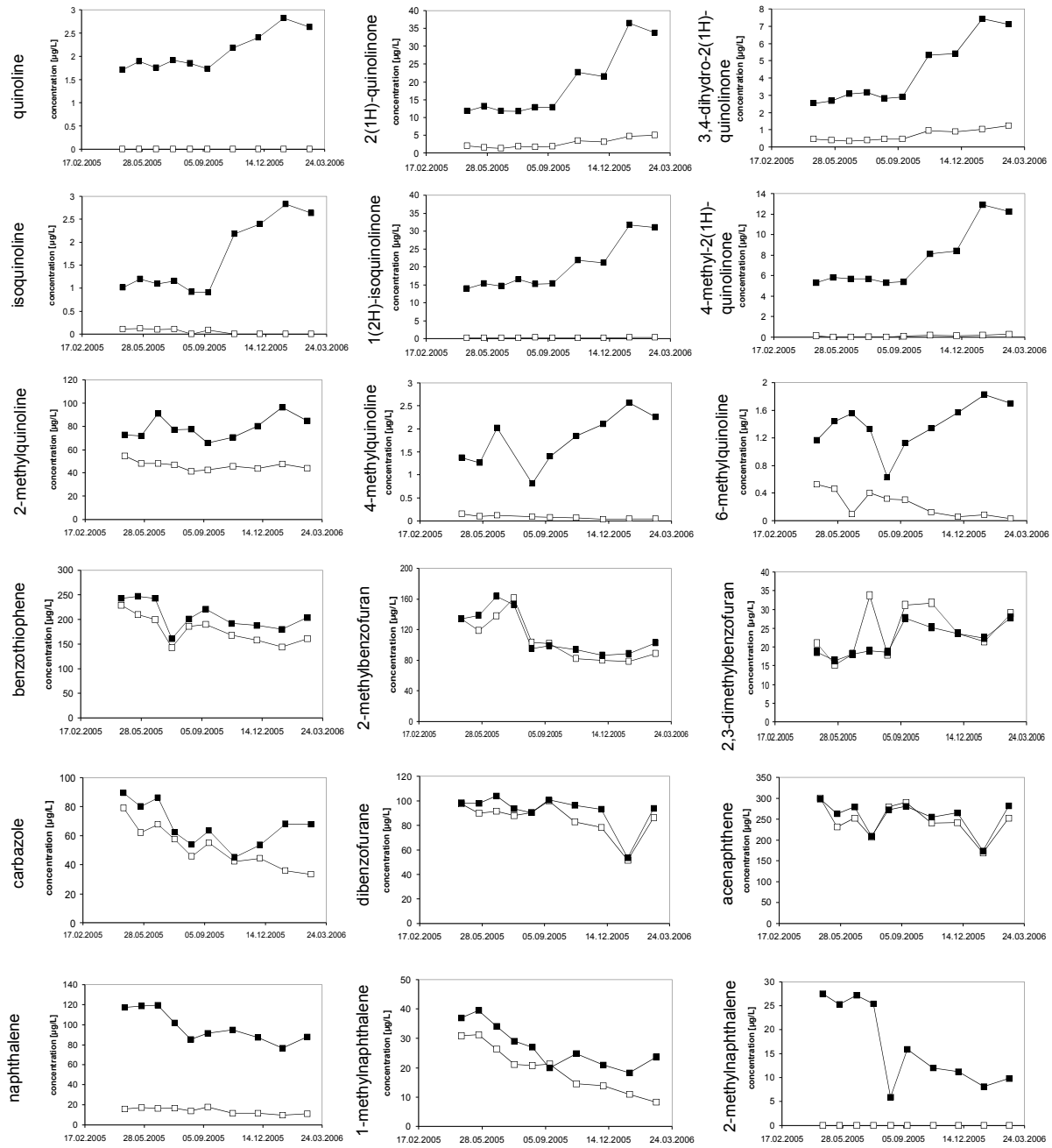


Figure 24: Comparison of concentrations of selected hetero- and homocyclic compounds in the on-site column (MP) of the Wülknitz site. Legend: concentrations in the biotic column (□) and the abiotic control column (■).

4.2.2.3 Microbial degradation in the aquifer

The understanding of *in situ* biodegradation processes, physicochemical gradients and distribution of individual redox species is crucial and not possible to achieve by conventional wells. These wells have filter units of about 1 m or even longer leading to the dilution of groundwater from above and below the plume during the sampling.

In this chapter results from a high-resolution multi-level well located in Düsseldorf-Flingern are presented. The well allows a high vertical resolution of up to 3 cm across the fringe and centre zones of the plume of contaminants and therefore allows a comparison of different contaminant plumes as well as a comparison with the plumes of electron acceptors. Therefore, a correlation of the plumes width and distribution of contaminants with those of electron acceptors may give some hints for microbial degradation.

High-resolution groundwater sampling led to distinct vertical concentration profiles of heterocyclic compounds, which allowed the detection of a plume thickness of 0.5-1 m. The sampling with conventional multi-level well (sampling depth: 6.5; 7; 8; 9; 10 m) may lead to an underestimation of concentration because of dilution by non-contaminated regions (Figure 25).

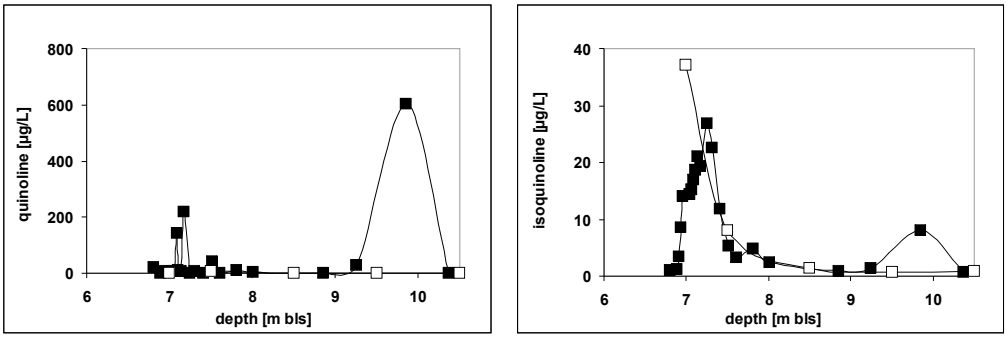


Figure 25: Comparison of vertical distribution of high-resolution well and conventional multi-level well. High-resolution multi-level well (■) and conventional multi-level well (□).

Results from two sampling campaigns showed similar results in general. Clear differences in compounds distribution were visible. Concentrations of most compounds decreased to levels near the limit of detection within the upper first meter of the saturated zone. Within the two campaigns of samplings (August 2006 and February 2007) the groundwater level dropped 24 cm. For all contaminants the level of concentrations over the size of the plume remained in a similar range. In the first campaign highest concentration of all compounds was present within a depth of 6.5-7 m. At the second sampling campaign the plume was dropped together with the groundwater level and highest concentrations were found correspondingly deeper.

It was shown in previous studies that sulfate- and iron-reduction are the predominant redox conditions associated with biodegradation at the Düsseldorf-Flingern site (Eckert, 2001; Wisotzky, 2000). The analyses of electron acceptors by Anneser et al. (2007) in samples from the high-resolution multi-level well supported the earlier findings: Highest concentration of dissolved iron were found in the core of heterocycles plume indicating the presence of iron-reducing conditions, while at the plumes fringe small-scale distribution of ferrous iron, low concentrations of sulfate and higher ones of sulfide suggest an overlapping of iron- and sulfate-reduction. In the plumes centre sulfate and nitrate were entirely depleted. Therefore, the range of 6.56-6.90 m (August) and 6.59-6.76 m (February) was characterized by high microbial activity, supported by decreasing pH-values as well as lower redox potential (Figure 27). A correlation with electron acceptors showed that compounds

investigated in this study were present in a depth of high microbial activity. Microbial degradation will cause differences in widths of compounds plumes. Therefore, a comparison of the distribution of different contaminants as well as the plumes width may give some indications for biodegradation, especially a comparison of compounds with similar physicochemical characteristics. The different widths of the plume are presented in Table 12.

Table 12: Width of the contaminants plume in August 2006 and February 2007.

compound	maximum concentration August/February [µg/L]	width of the plume in August 2006 [m]	width of the plume in February 2007 [m]
N-Heterocycles			
quinoline	218/310	0.77	0.50
isoquinoline	27/17	1.20	1.16
1-methylisoquinoline	3/2	0.72	0.65
2-methylquinoline	26/29	0.80	0.65
4-methylquinoline	4/1	0.70	0.55
6-methylquinoline	3/1	0.70	0.55
2(1H)-quinolinone	27/23	0.77	1.25
1(2H)-isoquinolinone	334/198	0.77	0.65
3,4-dihydro-2(1H)-quinolinone	25/11	0.44	0.55
2,4-dimethylquinoline	39/34	0.80	0.55
2,6-dimethylquinoline	3/3	0.70	0.45
9(10H)-acridinone	8.8/82	1.44	2.44
6(5H)-phenanthridinone	278/163	2.44	2.09
carbazole	93/211	3.31	2.64
S-Heterocycles			
benzothiophene	1276/1538	1.00	1.19
3-methylbenzothiophene	498/219	1.20	1.79
O-Heterocycles			
benzofuran	1245/896	0.80	0.65
2-methylbenzofuran	820/707	1.00	1.19
dibenzofuran	1807/1159	2.04	1.79
Homocycles/PAHs			
2-naphthol	66/20	0.80	0.73
2-methylnaphthalene	1239/1089	0.80	0.72
1-naphthol	78/26	0.90	0.87
indene	1882/3091	1.00	1.19
naphthalene	8108/5414	1.00	1.19
1-methylnaphthalene	1019/1197	1.00	1.49
indan	1192/817	1.20	1.05
phenanthrene	45/141	1.91	1.46
1,3-dimethylnaphthalene	161/233	2.04	1.46
fluorene	368/447	3.41	2.74
acenaphthene	1089/1358	3.56	3.09

Dependent on the compounds investigated the width of the plume differed from 0.4 to 3.56 m. For the most 2-ring-compounds clear formed plumes were detected, while for others two maxima were found. All 3-ring heterocycles analysed (carbazole, acenaphthene, dibenzofuran, fluorene) showed higher vertical distribution than the 2-ring compounds. The plume of these compounds was up to 2-3.56 m in contrast to a thickness of ~1 m for 2-ring compounds.

The vertical distribution of a broad number of compounds is shown in the following text or in Figure 54 and 55, appendix.

N-Heterocycles - parent compounds. In general, quinoline compounds were present within the plume of sulfide, which is the region of lowest sulfate concentration.

The comparison of quinoline distribution and width of the plume with its isomeric compound isoquinoline showed some interesting differences (Figure 26). For quinoline high variances in concentration were found, with maximum levels up to 200-300 µg/L at 6.59 and 6.70 as well as 6.81-6.83 m depth, respectively. The plume of isoquinoline was observed in a lower range of concentration (20-25 µg/L), but a higher vertical width of the plume (0.77/1.20; 0.50/1.16 m).

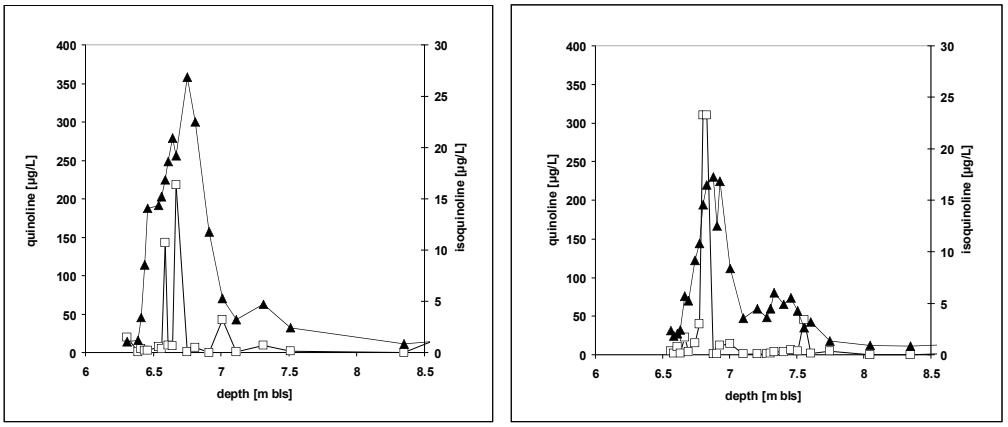


Figure 26: Comparison of quinoline and isoquinoline. Legend: quinoline (□), isoquinoline (▲) (left hand side: data from August 2006 and right hand side: data from February 2007).

N-Heterocycles - comparison of parent compounds and their hydroxylated analogs. Different vertical distribution of quinoline parent compounds and their hydroxylated analogs were determined. Quinoline and its hydroxylated analogs 2(1H)-quinolinone as well as 3,4-dihydro-2(1H)-quinolinone were measured. The distribution and range of concentrations showed high differences: In several depths quinoline was present in higher concentrations than its hydroxylated analogs. Data from both sampling campaigns showed that the hydroxylated compounds occurred in lower depths (maximum of 2(1H)-quinolinone plume: 6.41 m in August; 6.63 m in February) than the parent compound, whose maximum was located in a depth of 6.67 and 6.83 m. The hydroxylated compounds were mostly present in the fringe of the plume, i.e. in the region of highest sulfate-reducing activity. Furthermore, a second maximum of 2(1H)-quinolinone was found in higher depth than quinolines maximum in concentration. The plume width of 3,4-dihydro-2(1H)-quinolinone was smaller than that of 2(1H)-quinolinone at similar levels of concentration and showed only one maximum in concentration.

In all depths higher concentrations of 1(2H)-isoquinolinone were present than isoquinoline concentrations. The further reduced analog 3,4-dihydro-1(2H)-isoquinolinone was absent. However, isoquinoline compounds showed the same tendency in distribution as the quinoline compounds. Maximum of the hydroxylated isoquinoline plume was found at 6.39 m in August and 6.67 m depth in February, while the maximum of the isoquinoline plume was determined at 6.75 and 6.88 m depth, respectively.

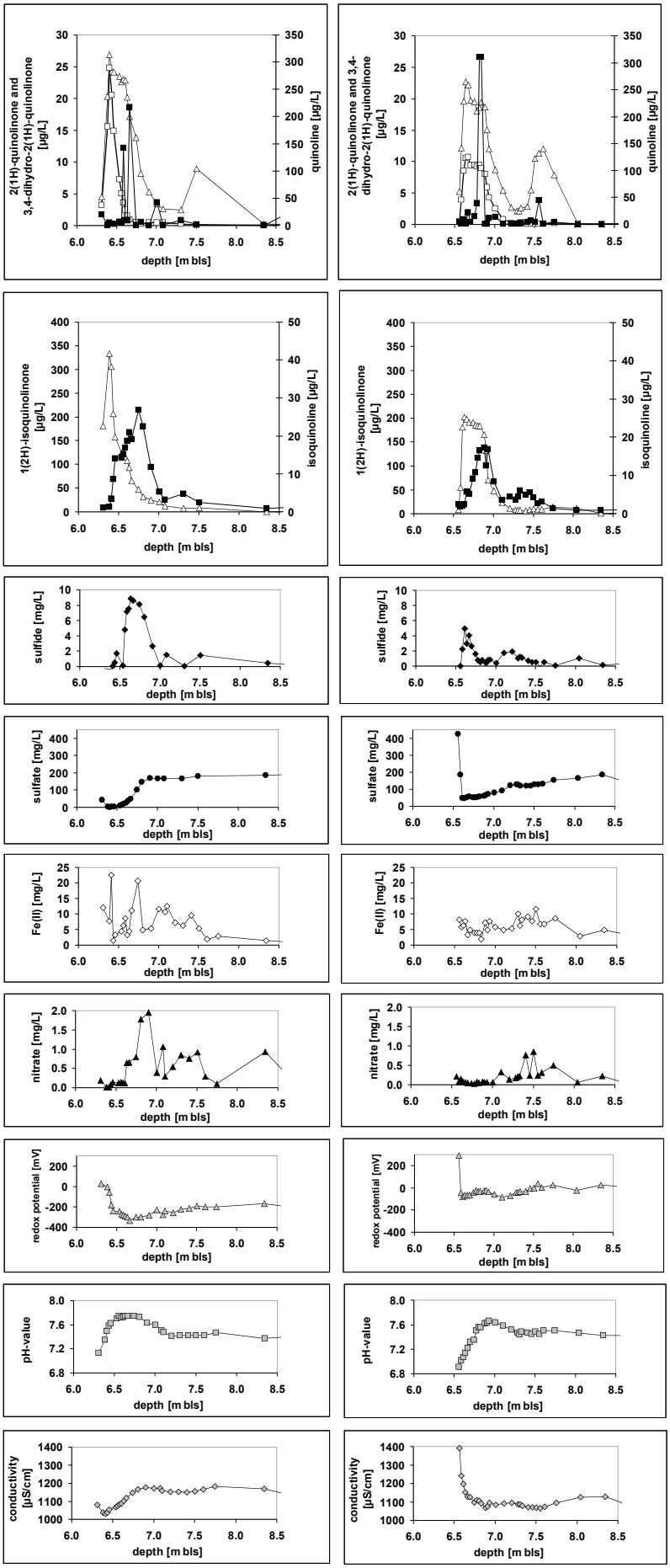


Figure 27: Vertical distribution of quinoline and isoquinoline compounds, electron acceptors sulfate, nitrate as well as reduced species: sulfide and Fe(II), redox potential, pH-value and conductivity. Left hand-side data from August 2006 and right hand-side from February 2007.

Legend: quinoline or isoquinoline (■); 2(1H)-quinolinone or 1(2H)-isoquinolinone (△); 3,4-dihydro-2(1H)-quinolinone (□); sulfide (◆); sulfate (●); Fe(II) (◇); nitrate (▲); redox potential (△); pH-value (▣); conductivity (◈).

Data of electron acceptors, pH-values, redox potential and conductivity were provided by B. Anneser and C. Griebler, GSF, Neuherberg.

Methylsubstituted quinolines. A comparison of methylquinoline isomers in the plume gave some additional indications for differences in microbial degradation. There were high differences found in concentration levels of methylquinolines in both field campaigns. 2-Methylquinoline was detected in highest concentration (20-30 µg/L) in contrast to all the other methylquinolines (1-4 µg/L) present. At different level of concentration the width of the plumes of 2-methyl- and 1-methylisoquinoline were found to be similar. But both plumes were more expanded than the plume of 4- and 6-methylquinoline (Figure 28). For 2-methylquinoline the decrease in concentration with depth was less than that of other methylquinoline compounds.

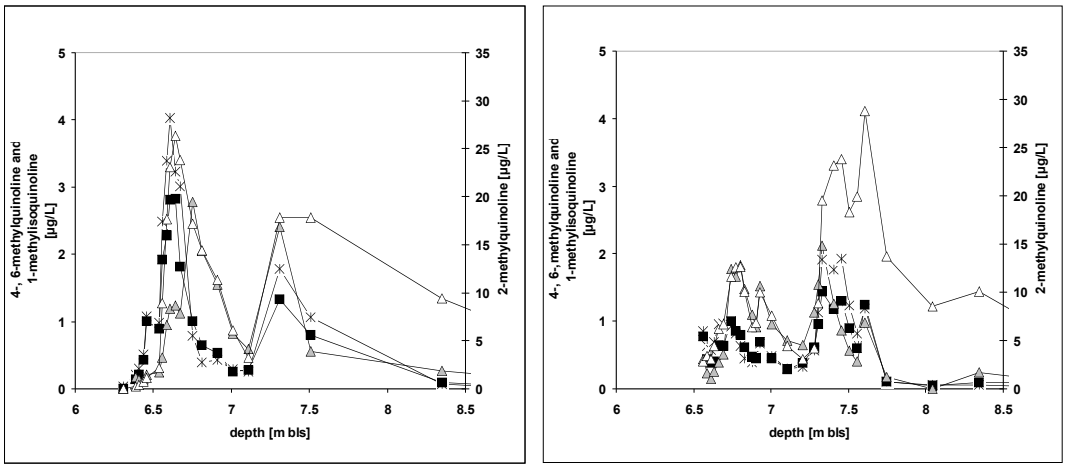


Figure 28: Comparison of the plumes of methylquinolines (2-, 4- and 6-methylquinoline as well as 1-methylisoquinoline). Legend: left hand-side: data from August 2006; right hand-side: data from February 2007. 4-methylquinoline (∗); 6-methylquinoline (■); 1-methylisoquinoline (◬); 2-methylquinoline (△). Note differences in y-axis!

In addition to high differences in the level of concentration, the distribution of 2,4-dimethyl- and 2,6-dimethylquinoline highly differed (Figure 29). The ratio of 2,4-dimethylquinoline/2,6-dimethylquinoline generally was found to be low in August, while in a depth of 6.5 m a high increase of the ratio was found. In February the ratios were the same within a broader region (6.5-7 m) with little increase in a depth of 8.5 m. Ratios were highest in the fringe of the sulfide plume, especially in August 2006, where the ratio was highly exceeded in two samples.

As has been observed for quinoline and isoquinoline parent compounds and their hydroxylated analogs, highest concentrations of 4-methyl-2(1H)-quinolinone were found in the plumes fringe in August, while this finding was merely clear in February. However, at both sampling times concentrations of 4-methyl-2(1H)-quinolinone were found a factor ~30-40 higher than that of 4-methylquinoline. These findings were also true for the sum of methylquinolines and methyl-2(1H)-quinolinones. Also methyl-2(1H)-quinolinones and methylquinolines in sum showed the highest concentrations of the hydroxylated compounds within the methylquinolines fringe in a depth of 6.39/6.64 m (maximum August/February), while the maxima of the plumes of methylquinolines were present in 6.61/6.81 m (Figure 53, appendix).

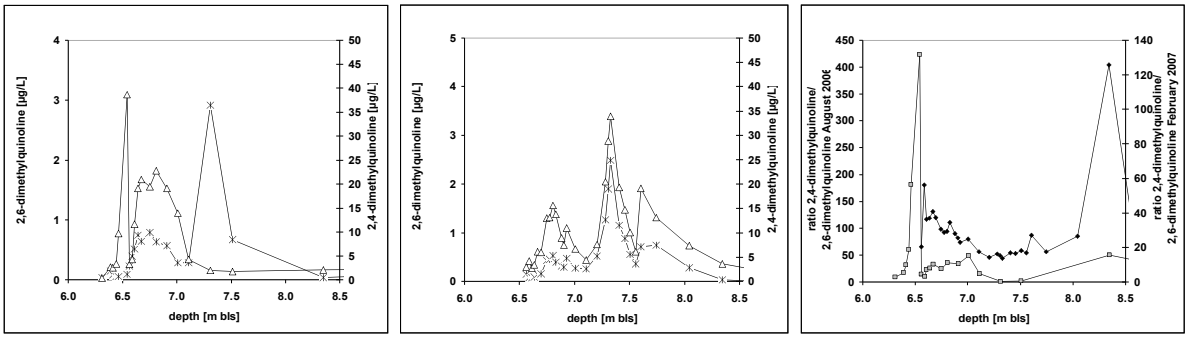


Figure 29: 2,4-Dimethyl- (∗) and 2,6-dimethylquinoline (△); (A) August 2006; (B) February 2007; (C) ratio of 2,4-dimethylquinoline/2,6-dimethylquinoline at the two sampling campaigns; grey August 2006; black February 2007.

S- and O- Heterocycles. Concentration levels of benzothiophene and benzofuran were found to be similar at both sampling times. When comparing the distribution in the plume a smaller width was determined for benzofuran (0.80 m in August/0.65 m in February) than for benzothiophene (1.00 m in August/1.19 m in February). Similar results were obtained comparing benzofuran with its methylated analog 2-methylbenzofuran. Even though the concentration of 2-methylbenzofuran was much lower than benzofuran, a stronger decrease was detected in the fringe zone of benzofuran.

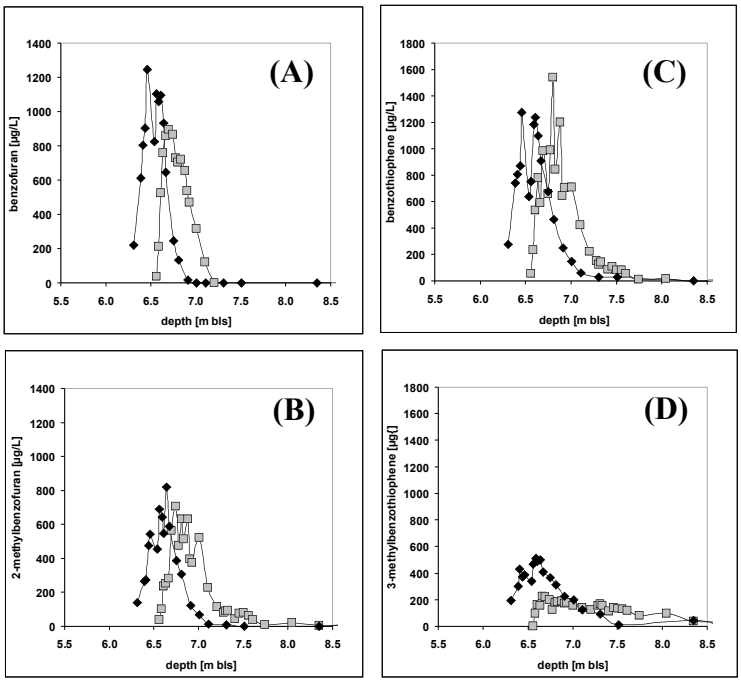


Figure 30: Plumes of O-heterocycles benzofuran (A) and 2-methylbenzofuran (B) as well as S-heterocycles benzothiophene (C) and 3-methylbenzothiophene (D). Legend: plumes of August 2006 (◆) and of February 2007 (■).

When inspecting the plume width of benzothiophene and 3-methylbenzothiophene similar tendency of distribution is obvious. The width of the plume of 3-methylbenzothiophene was found about 20 cm broader, although the level of concentration was about a factor of 2 lower.

Homocycles/PAHs. Naphthalene was determined as the major PAH present in the plume, in concentrations up to 8 mg/L, while 1- and 2-methylnaphthalene were both detected in concentrations up to 1.2 mg/L and 1,3-dimethylnaphthalene up to 250 µg/L. The width of the plume of naphthalene was about 1 m in August 2006 and 1.19 m in February 2007. At both sampling times the naphthalene plume was broader than the 2-methylnaphthalene plume. In contrast the 1-methylnaphthalene plume was identical to the naphthalene plume in August 2007 at lower level of concentration but showed a broader plume in February 2007. The plume of 1,3-dimethylnaphthalene was broader at both sampling times.

Comparing the vertical distribution of methylated naphthalenes some differences were evident. The plume of 1-methylnaphthalene was found to be slightly broader (1 m in August/ 1.49 m in February) than the plume of 2-methylnaphthalene (0.8 m in August/ 0.72 m in February) in both sampling campaigns. The 1,3-dimethylnaphthalene plume showed a width of 2.04 m in August and 1.46 m in February at a lower level of concentration (Figure 31).

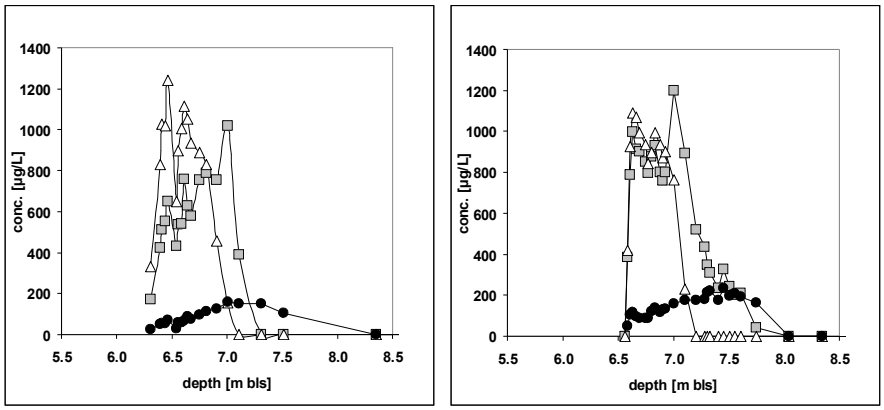


Figure 31: Plumes of methylated naphthalenes: 1-methylnaphthalene (□); 2-methylnaphthalene (△); 1,3-dimethylnaphthalene (●); left: August 2006 and right: February 2007.

Even if the level of concentrations of indan and indene differed, the width of the plume remained constant within both samplings and was comparable to the plume of naphthalene (Figure 55, appendix).

Hydroxylated compounds. 1-Naphthol and 2-naphthol showed similarities in their level of concentration and distribution within depth. However, the plume of 1-naphthol was found to be slightly ~10 cm broader. The highest concentrations were detected within the region of the sulfide fringe.

For 9(10H)-acridinone high differences in maximum concentrations were found comparing the two sampling campaigns, while its analog 6(5H)-phenanthridinone – with the exception of one data point - was detected in the same range of concentration. In August 9(10H)-acridinone was distributed over a high range of depth, while concentrations were low. In February clear maximum concentrations were reached. The plume of 6(5H)-phenanthridinone was located in lower depths than the plume of 9(10H)-acridinone. The parent compounds acridine and phenanthridine were absent. Data showing the compounds distribution of these hydroxylated compounds are shown in appendix in Figure 54, appendix.

4.2.3 Site directed analyses: Discussion and conclusion

The complete set of results on the occurrence and distribution of NSO-heterocyclic compounds at the field sites, the inventory, the data of the microcosm and the on-site column experiments as well as observations from the high-resolution well analyses will be discussed in the following paragraph aiming to work out indications for microbial natural attenuation processes in the field.

The site survey allows the long distance comparison of distribution behaviour of various compounds. A rough estimation of the fate of the contaminants in the aquifer was possible with this approach. However, to reach meaningful conclusions on mass balance, a well-structured field site with a high number of wells in the in- and out-flow, which are directly behind each other in the groundwater flow direction, was found to be necessary. This requirement was achieved at the Castrop-Rauxel site, while the wells at the Düsseldorf-Flingern site were more distributed over the site and not laying clearly in line of the groundwater flow direction. For data of the Wülknitz site no calculations of mass loads were possible, because of the limited number of wells, distributed over high distances and furthermore, known changes in groundwater flow direction. However, a rough comparison of compounds decrease was done.

In addition to these limitations concerning the distribution of wells for sampling, heterogeneity in the aquifer will influence the transport of chemicals in the plume. In addition, more than one source might be present so that a comparison of compounds distribution may be problematic. Various parameters (dilution, volatilization, sorption, degradation) influence the fate of compounds in the field site. Only biological degradation is a destructive parameter leading to long term decrease of the contamination plume.

The microcosm and on-site column studies show the decrease in concentration within a closed system and a time period. The experiments produced information on the microbial degradation under different redox conditions and may indicate the most active process. By usage of controls, where the microbes have been inactivated, a transformation could clearly be attributed as the action of bacteria and allowed to distinguish the degradability of different chemicals. Most important, the approaches allowed to observe the decrease of compounds and the simultaneous formation of metabolites. Generally, in column experiments transformation/degradation rates were higher than in diluted microcosms supplemented with populations in the groundwater. Even after a short period of 4 weeks, differences in biotic and abiotic columns were found, which can be explained by the fact that aquifer material is colonized on the surfaces by higher population densities than those present in the groundwater.

The high-resolution well allowed the micro distance comparison of concentration of various compounds. Heterogeneity is more or less absent on this scale. The decrease of a compound resulting in the accumulation of a metabolite is a more clear result than in the site survey. The presence of natural attenuation can be seen easily by the comparison of compounds with similar physicochemical characteristics.

Interpretation of field data in context to natural attenuation

The possible parameters influencing the distribution of different compounds at the three investigated sites are discussed in the following.

At the three sites investigated generally a high decrease in contaminant concentration within a distance of 40-250 m was found. A rapid decrease in contamination level within a distance

of about 100-200 m is an often-described phenomenon at tar oil contaminated sites (Johansen et al., 1997b; Kiilerich and Arvin, 1995; Zamifirescu and Grathwohl, 2001).

A rough estimation shows that the plume size at all of the three sites has to be much larger if only the flow rate is included into the calculation of the formation of the plume and if attenuation processes are neglected. Even if a high retardation factor is included the remaining difference between calculated and actual plume size can only be explained by the presence of biodegradative NA-processes at the sites investigated. This rationale is seen in detail for the Castrop-Rauxel site: the groundwater velocity at the site is about 31 m/a. If the retardation is only about a factor of 3, then the solutes should have travelled, in the absence of degradation, at least in the order of 10 m/a. The tar oil has impacted the groundwater at least since the Second World War, then the solutes had on the order of 65 years to travel from their point of release. Thus we should expect a plume at least of about 650 m long instead of the known size of 325 m in maximum, unless something prevented it, i.e. *in situ* degradation.

The broad number of compounds investigated shows some interesting variances in compounds decrease within the field sites. Dilution occurs for all compounds at the same extent and the impact of volatilization on the dissolved contaminant reduction is generally assumed to be negligible (Wiedemeier et al., 1999). Processes which have to be considered for these observations are biodegradation as well as sorption. Sorption of organic compounds in the sediment or soil is often found as a function of the organic carbon-content. Bi et al. (2006, 2007) studied sorption of several heterocyclic compounds towards five eurosoils with different content of organic carbon. For non-ionizable heterocyclic compounds they found high correlation of sorption with the organic carbon content showing the predominance of hydrophobic sorption. Sorption at the tar oil contaminated sites may be estimated as a minor process because of the low organic carbon content and gravel, sandy structure of the aquifers. However, for the evaluation of differences in distribution of compounds at the field site, log P_{OW}-values should be considered. Any difference in decrease over the field site of non-ionizable compounds with similar physicochemical characteristics, i.e. exhibiting similar log P_{OW}-values (Table 22, appendix) can clearly be explained by variations in biodegradability of contaminants compared.

The analyses of groundwater at micro-scale allowed to study the simultaneous presence of heterocyclic and homocyclic compounds within the plume at the depth where there is a leak of electron acceptors. BTEX and PAH compounds analysed by Anneser et al. (2007) were found in identical depths as the heterocycles and homocycles analysed here. A high decrease in concentration of electron acceptors (here sulfate) within the plume seems to be a result of the presence of microbial degradative processes and the restriction of transversal dispersion of electron acceptors from the peripheral region into the centre of the plume (Anneser et al., 2007). Therefore, microbial degradative processes are limited within the plumes centre and biodegradation may occur mostly at the plumes fringe as shown graphically in Figure 32. The decrease of sulfate and increase of sulfide indicates the presence of the region in the plumes fringe, which exhibits highest microbial activity. The following considerations may indicate differences in biodegradability of organic compounds:

(1) Differences in microbial degradability of compounds with similar physicochemical properties may result in differences in the plumes width: this might be true for isomers like quinoline/isoquinoline or 1-methyl-/2-methylnaphthalene.

(2) A non-substituted compound will form a higher vertical dimension of the plume compared to the methylsubstituted analog because of its higher water solubility and higher dissolution from tar oil if both are present in similar concentrations within the

contamination source. Only compounds of similar concentration levels in the water phase may be identical in plumes width without differences in biodegradation. A broader plume size of a compound present in lower concentration indicates less degradability in contrast to a compound found in higher concentration forming a smaller plume width.

(3) The presence of metabolites within the plumes indicates microbial activity in the region of the plume, where electron acceptors are accessible.

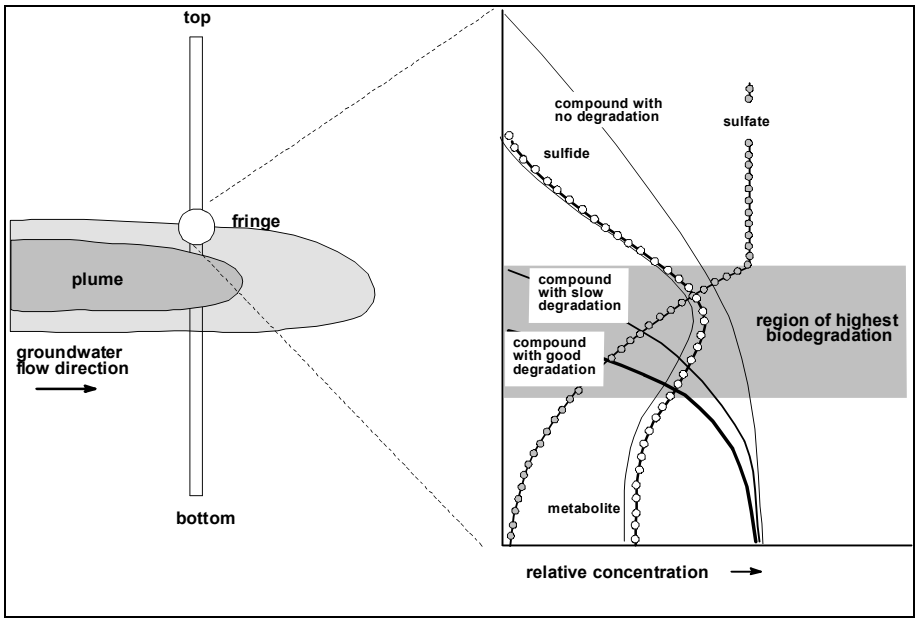


Figure 32: Characterization of the vertical resolution of the plume and concentration profiles in the fringe region.

4.2.3.1 Implications for natural attenuation

The decrease in concentration along the plumes together with the results from the more artificial degradation experiments and the high-resolution well led to the general conclusion that biodegradative processes towards heterocycles and homocycles are present in the aquifers. The discussion will include the evaluation, which heterocyclic component of a tar oil indicates natural attenuation properly. Which differences in biodegradability might be deduced from the behaviour of the different groups of components in the field and the microcosms?

Quinolines. The isomers quinoline and isoquinoline behave very different in the field sites. Quinoline enters the testfield in Castrop-Rauxel in amounts about a factor 10 higher, it is decreased almost completely within a distance of 36 m, while isoquinoline is decreased less. The same is true for the Düsseldorf-Flingern and the Wülknitz site, where isoquinoline was found more often and was more widespread distributed than quinoline. A plausible explanation for these findings are differences in biodegradation of these isomers.

These findings were supported by results from the high-resolution multi-level well. The high variations in quinoline concentrations as well as narrow width and unshaped quinoline plume in comparison to a broader one with lower concentration of isoquinoline indicates a higher microbial degradation rate towards quinoline than isoquinoline.

However, in microcosms with groundwater as well as in the column experiments no differences in degradability of these isomers could be determined, since the concentrations of quinoline and isoquinoline were decreased up to 100 % in the time period tested. Independently on the redox conditions tested high transformation rates of these compounds were found. Only in the microcosms containing quinoline and isoquinoline as minor compounds (<1 % of hydroxylated quinoline compound) transformation was absent or a very slow process.

These findings are in accordance to results presented in the literature. Transformation of isoquinoline was found to be a slower and incomplete process in comparison to the transformation of quinoline which was mostly fast and complete (Pereira et al., 1987; Johansen et al., 1997a).

Hydroxylated quinolines. In the groundwater of all of the three sites hydroxylated quinoline compounds, 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone and 1(2H)-isoquinolinone, were found in high concentrations up to the mg/L-range and were present in obviously higher concentrations than the non-substituted parent compounds quinoline and isoquinoline. In recent literature these hydroxylated analogs are described as metabolites of the anaerobic transformation of quinoline and isoquinoline (Pereira et al., 1987; Johansen et al., 1997a,c; Liu et al., 1994a,b). The presence of these hydroxylated compounds in groundwater may be discussed in context to microbial formation, while their presence in original tar products has to be kept in mind as discussed in chapter 4.3.3. However, indications for their microbial formation are as follows: studying the distribution of parent compounds, quinoline and isoquinoline, and their hydroxylated analogs, similar distribution patterns were seen for 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone and quinoline as well as for the isomeric compounds 1(2H)-isoquinolinone, 3,4-dihydro-1(2H)-isoquinolinone and isoquinoline at the sites with sufficient number of wells, which were not found for other compounds investigated like other quinoline compounds as well as S- and O-heterocycles.

The differences in size and vertical distribution of the plumes analysed by the high-resolution well in Düsseldorf-Flingern showed that maximum concentrations of hydroxylated compounds present, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone, are located in the fringe of the parent compound quinoline. The isomers isoquinoline and 1(2H)-isoquinolinone showed the same distribution pattern. Therefore, the maximum concentration of hydroxylated analogs was present in a region, which is found to exhibit high microbial activity. These results may indicate the formation and accumulation of the metabolites due to a slow transformation of the hydroxylated quinolines.

Comparing the degradative behaviour of parent compounds and hydroxylated analogs in degradation experiments high differences were found. In some groundwater samples from the Wülknitz site the accumulation of 2(1H)-quinolinone as well as 2(1H)-isoquinolinone was detected. The absence of any transformation of metabolites was observed: 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone as well as 2(1H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone persisted over a long period of time in some microcosms. Similar results were obtained with the groundwater of the Düsseldorf-Flingern site, where the hydroxylated metabolites were only transformed further under particular conditions, but only in minor amounts.

Different results were obtained with some samples of the groundwater from Wülknitz as well as the on-site column, where a decrease of 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone as well as 2(1H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone of up to 100 % was observed. An explanation for these diverse results might be the following:

In samples with high concentrations of quinoline or isoquinoline (like IW1 ~1100 and 180 µg/L, respectively) high transformation rates of the compounds were present. Due to this situation, the transformation of the hydroxylated compounds is inhibited or superposed by the formation of hydroxylated compounds. Therefore, a decrease of hydroxylated metabolites is not detectable. In contrary, in those samples, where quinoline and isoquinoline were absent or present in only low concentrations, the determination of the transformation of 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone, 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone was possible.

3,4-Dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone, which were detected in the groundwater of all of the 3 sites, have never been determined in tar oil contaminated groundwater in the past. Concentrations especially of 3,4-dihydro-2(1H)-quinolinone were considerable in the Castrop-Rauxel testfield. While 3,4-dihydro-2(1H)-quinolinone is a known metabolite of the anaerobic quinoline degradation, 3,4-dihydro-1(2H)-isoquinolinone was not detected as metabolite until now.

The large differences in concentration between parent compounds and respective hydroxylated derivatives were also detected at other creosote or tar oil contaminated sites (Pereira et al., 1987; Johansen et al., 1997b). In all studies quinoline and isoquinoline were detected in obvious smaller amounts compared to high concentrations of 2(1H)-quinolinone and 1(2H)-isoquinolinone. Therefore, high activity of formation of hydroxylated quinoline compounds was indicated or the other way round, low degradative potential of these compounds was present.

These findings show that the exclusive quantification of the parent compounds may lead to an underestimation of the amount of N-heterocycles present in the tar oil contaminated groundwater.

Methylquinolines. For methylquinoline isomers a decrease in high amounts over the field sites was found. Differences in decrease over the field sites may give some clear indications for differences in biodegradation of the methylsubstituted isomers. At the Castrop-Rauxel and Düsseldorf-Flingern site 2-methylquinoline was found as the methylquinoline present in highest concentrations. However, it was not possible to evaluate differences in decrease over the distance of the field sites for 2-methylquinoline and 1-methylisoquinoline in comparison to the other isomers at the both sites. Decrease in concentration of all isomers through the field site was found to occur at an identical degree. At the Wülknitz site, a tendency of minor decrease of 2-methylquinoline and 1-methylisoquinoline was indicated.

When comparing the plumes of methylsubstituted quinolines in the high-resolution well, higher microbial degradation of 4- and 6-methyl isomers was indicated in August 2006. Only 2-methyl- and 1-methylisoquinoline plumes were characterized by a broader distribution within a depth of up to 7 m. The ratio of 2-methylquinoline to the sum of all other methylquinolines increased with increasing depth. These findings may only be explainable by lower microbial degradative activity towards the 2-methylquinoline and 1-methylisoquinoline isomer.

The microcosm as well as the on-site column experiments showed that all methyl(iso)quinolines studied were transformed. Interestingly, also concentrations of 2-methylquinoline and 1-methylisoquinoline decreased in the microcosms as well as in the on-site columns. 2-Methylquinoline transformation was mostly found to occur at a lower percentage, but initial concentration was about a factor of ~10 higher than concentrations of the other methylquinoline isomers. Therefore an important decrease took place.

These findings were surprising. From the literature (Johansen et al., 1997a,c; Pereira et al., 1987; Liu et al., 1994b; Figure 4) on degradation of methylquinolines, degradation of 2-methylquinoline and 1-methylisoquinoline was not expected. The degradative pathway of quinoline compounds was found to start with a hydroxylation in position 2 (position 1 for isoquinoline compounds). Therefore, the degradation or transformation of quinoline compounds substituted by a methylsubstituent in the prominent position was blocked, as has been shown when the degradation of the pure chemicals was studied as sole source of carbon. The results presented here indicate that the initial microbial attack on 2-methylquinoline and 1-methylisoquinoline in contaminated groundwater includes other primary steps, which probably may be discussed in context to cometabolic transformation. However, these transformation processes may be slower than transformation of other methylquinolines resulting in the differences in distribution at the filed sites. Methylation and hence formation of methylquinolines as a degradative step may also be a possible explanation for differences in distribution of isomers, which is described in the anaerobic degradation pathways of other aromatic compounds (Safinowski and Meckenstock, 2006; Martus and Püttmann, 2003).

Dimethylquinolines. Dimethylquinolines were found at the field sites in similar concentrations than methylquinolines and were also more widespread distributed than the non-substituted parent compound quinoline. A decrease over the distance of the sites was comparable to methylquinoline isomers.

2,4-Dimethyl- and 2,6-dimethylquinoline were not transformed in every microcosm with groundwater as well as in the on-site columns. Differences in distribution found in the high-resolution multi-level well showed some variances of the ratio of 2,4-dimethyl-/ 2,6-dimethylquinoline. Increasing values of the ratio found at the plumes fringe may be discussed as indication for a better degradability of the 2,6-compound. Furthermore the increase of ratio may also be explained by a higher formation of the 2,4-dimethyl-analog from non-substituted parent compound or methylquinolines. The same consideration may be true for the Wülknitz on-site column indicating an increase in concentration of 2,4-dimethylquinoline due to formation.

Hydroxylated methylquinolines. A broad spectrum of methyl-2(1H)-quinolinones was found at the sites, which were present in high concentrations, especially near the source of contamination in Castrop-Rauxel and Wülknitz. Using NMR-techniques these compounds were characterized as methyl-2(1H)-quinolinone isomers.

According to the non-substituted quinoline compounds the hydroxymethylated compounds were present in higher concentrations than the methylated parent compounds. Also in the high-resolution well the concentrations of methyl-2(1H)-quinolinones were determined at much higher concentration than concentration of methylquinolines. As has been found for non-substituted quinolines, the hydroxylated compounds were present in highest concentrations within the methylquinolines fringe indicating formation and/or less degradability of hydroxylated analogs. Furthermore, less decrease of the hydroxylated methylquinolines within the field sites was indicated, maybe due to intermediary formation. This tendency was also detected in degradation experiments and may be due to the formation and accumulation of hydroxylated compounds in the meantime.

Other N-heterocyclic compounds. At the three sites carbazole was mainly detected in the region of highest contamination, possibly due to its lower water solubility and a higher sorption characteristics than 2-ring compounds.

Microbial transformation of carbazole was determined in the microcosms as well as in the on-site columns. The microcosms supplemented with groundwater from Düsseldorf-Flingern or Wülknitz showed highest degradation rates under iron-reducing conditions, while the decrease in concentration was slow or absent under sulfate-reducing conditions. Some minor degradation was observed in the denitrifying microcosms. Until now a degradation of carbazole was described to occur under methanogenic conditions (Pereira et al., 1987) while in the studies of Liu et al. (1994a) and Dyreborg et al. (1997) investigating denitrifying, sulfate-reducing and methanogenic conditions, degradation of the compound was not observed. In these studies the degradation was also tested with aquifer material. A reason for the lack of degradation of carbazole was thought to result from the high sorptive characteristics leading to missing bioavailability (Liu et al., 1994a). However, results from the on-site column experiments showed the degradation in presence of aquifer material, but in comparison to quinoline degradation, the decrease of carbazole was much lower. Results from the high-resolution well showed that carbazole was more distributed into the deeper ground in comparison to 2-ring compounds, although the level of concentration was lower. Less degradability and less transport with the groundwater may be the reason for an expanded plume with depth.

6(5H)-Phenanthridinone, the oxygenated product of phenanthridine, showed high persistence in the degradation experiments. At the field sites 6(5H)-phenanthridinone was determined in the well of the highest contamination, but also downstream the plume. 9(10H)-Acridinone was only detected in several samples at the Düsseldorf-Flingern site, but the non-hydroxylated compound acridine was not determined. In comparison to its analog, 9(10H)-acridinone was detected in less wells, while it also was present in the same distance downstream the plume. In analogy to hydroxylation of quinoline compounds both compounds may be products of microbial degradation, which accumulate because of a higher persistence compared to their parent compounds, acridine and phenanthridine, as has been observed for quinoline compounds. However, both components 6(5H)-phenanthridinone and 9(10H)-acridinone may also be constituents of the original contamination (see results of the analyses of different tar oils in Table 45, appendix; Lang and Eigen, 1967).

S-Heterocycles. Benzothiophene was detected in high concentrations near the source of contamination as well as downstream the plume at all of the sites investigated, while a decrease of benzothiophene concentration was observed in all of the three lines of degradation experiments.

The transformation of benzothiophene was found in the present study under anaerobic conditions but is rarely described in the literature (Mundt et al., 2003; Kim et al., 1990). Until now, enrichments failed in which benzothiophene functions as sole source of energy and carbon. Up to now, only a cometabolic transformation of benzothiophene was reported, leading to the formation of carboxylated as well as carboxylated and reduced benzothiophene derivatives (Annweiler et al., 2001). Since the degradation of benzothiophene observed was a significant process in the microcosms, one has to assume that cometabolic processes may be of importance at the investigated sites.

To work out indications for natural attenuation by compounds distribution at the field site, log P_{OW}-values describing physicochemical characteristics were included into considerations. Higher log P_{OW} of benzothiophene (log P_{OW} = 3.12) indicates a higher hydrophobic sorption than expected for benzofuran (log P_{OW} = 2.67). Bi et al. (2006, 2007) when studying sorption to five eurosoils always observed a higher sorption of benzothiophene than benzofuran independent on composition of soil. However, Broholm et al. (1999) reported sorption to

specific inorganic sites as a relevant sorption mechanism for benzofuran. Therefore, the findings in the field, showing a widespread distribution of benzothiophene and even the presence in important amounts downstream the plume, may indicate minor degradability of benzothiophene compared to benzofuran, but also higher sorption of benzofuran may effect these differences.

In addition, methylsubstituted analogs of benzothiophene were determined in the groundwater of the sites and were still present downstream. They were absent in the microcosm experiments as well as in the groundwater of the on-site columns. However, the results of the plume width in the high-resolution well indicated less degradation of the methylsubstituted compound compared to the parent compound. In addition to poor degradability of methylbenzothiophenes, methylation has to be considered as a step leading to the formation of these compounds from non-substituted parent compounds (Safinowski et al., 2006; Martus and Püttmann, 2003).

The 3-ring analog dibenzothiophene, known as a typical tar oil component, was detected in few groundwater samples at the Düsseldorf-Flingern site, while it was not determined in groundwater of the Castrop-Rauxel and Wülknitz site. In Düsseldorf-Flingern dibenzothiophene was only detected in the first and second line of wells. In those wells, where dibenzothiophene was detected, it was present in concentrations similar to those of benzothiophene. Hence, higher decrease of dibenzothiophene in comparison to benzothiophenes was indicated.

O-Heterocycles. The comparison of the O-heterocycles benzofuran, methylbenzofuran and dimethylbenzofuran gives some hints for differences in their biodegradability.

Benzofuran entered the testfield in Castrop-Rauxel in high amounts (2.4 kg/a) in contrast to 2-methylbenzofuran with 1.1 kg/a, and 2,3-dimethylbenzofuran with 0.2 kg/a. Its decrease was found to be the highest (94 %), followed by 2-methylbenzofuran (80 %) and finally 2,3-dimethylbenzofuran with 68 %. In accordance, benzofuran was mainly found in the highest contaminated region at the Düsseldorf-Flingern as well as at the Wülknitz site, and it was eliminated in high amounts over the field sites. While the concentrations of benzofuran and methylsubstituted benzofurans in the highest contaminated groundwater were similar or the concentrations of benzofuran were even higher, the methylated benzofuran was found to be more widely distributed over the sites and was also present at a high distance from the source. The same was true for 2,3-dimethylbenzofuran which was found to be still present downstream the plume at all sites. Furthermore, the presence of some other methyl- and dimethylsubstituted benzofurans was indicated. Also at another tar oil contaminated gasworks site the predominance of these methylbenzofurans was determined downgradient (Zamifirescu and Grathwohl, 2001).

In microcosms the degradation of benzofuran was found to occur to much degree than degradation of 2-methyl- and 2,3-dimethylbenzofuran. The dimethylsubstituted benzofuran was found to be worse degradable than 2-methylbenzofuran. In the Wülknitz on-site columns benzofuran was absent, maybe due to microbial degradation over the distance from the contamination source to well 17/03 which groundwater was used in the on-site columns. 2-Methylbenzofuran was eliminated up to 8 % in the columns, while there was no decrease of 2,3-dimethylbenzofuran detectable.

Studying the plumes width in the high-resolution well in Düsseldorf-Flingern, 2-methylbenzofuran showed a broader plume than benzofuran which might also indicate higher degradability of benzofuran.

Sorption experiments of Bi et al. (2006) clearly indicated the increase in sorption by the presence of a methylsubstituent in O-heterocyclic compounds when comparing compounds of the same class. Therefore, sorption of methylated and dimethylated analogs is expected at higher value, because of the lower polarity shown by log P_{OW}-values of 3.2 and 3.6, respectively, in contrast to the log P_{OW}-value of benzofuran at 2.7. Therefore, the higher percentage in decrease over the field sites as well as a high amount eliminated of the less sorbing compound benzofuran gives a clear hint for a better biodegradability or, the other way round, less biodegradability of methyl- and dimethylbenzofurans in the field sites.

As another parameter, explaining the predominance of methylated benzofurans, microbial methylation reactions have to be considered, too. As it was discussed for other organic compounds (e.g. naphthalene or benzoates) (Safinowski and Meckenstock, 2006; Martus and Püttmann, 2003; Schmitt et al., 1996) the methylation may also be a step of microbial transformation process. Therefore, studies of methylation of heterocyclic compounds should be included into studies in the future.

In most microcosms minor degradation of the 3-ring analog dibenzofuran was observed, while in samples of the Düsseldorf-Flingern site this compound persisted completely. Furthermore, it was still detectable in groundwater samples downstream the plume. Also the presence of methylsubstituted dibenzofurans downgradient the Düsseldorf-Flingern site shows their high persistence or microbial formation.

PAHs/Homocycles. Within the 16 EPA-PAHs naphthalene, acenaphthene as well as fluorene represent those PAHs exhibiting highest water solubility and therefore were present in highest concentrations in the groundwater samples.

Concentrations of naphthalene were often much higher then those of other PAHs. Strong decrease in concentration over the three investigated field sites occurred. However, the compound was often detected downstream the plume because of high initial concentration. In all degradation studies naphthalene was the compound showing the highest decrease in concentration. The plume of naphthalene was relatively narrow, even though the concentration in the centre was high as has been shown in the high-resolution well. Degradation of 2-methylnaphthalene seems to be as good as degradation of naphthalene, or even better, while a clear statement is complicated because of high differences in initial concentrations. In the literature there is strong evidence that the initial step of naphthalene degradation is the methylation in position 2. Safinowski and Meckenstock (2006) reported that the sulfate-reducing pure culture N47 was able to grow on 2-methylnaphthalene without any lag phase, while the degradation of naphthalene occurred only after an induction period demonstrating the formation of an additional catabolic enzyme.

A comparison of the isomeric compounds 1-methyl- and 2-methylnaphthalene suggests that the degradation of 2-methylnaphthalene occurs at much higher rate than that of its analogs 1-methylnaphthalene. Results from sampling all over the field sites showed a higher decrease of 2-methyl- than 1-methylnaphthalene. Both isomers enter the testfield in Castrop-Rauxel in same amounts, but the decrease of 2-methylnaphthalene was found higher. The same was obvious from data of the Düsseldorf-Flingern as well as the Wülknitz site. Furthermore, this indication was evident in the complete set of experiments on the degradation, i. e. in the microcosms and in the on-site columns of the Wülknitz-site as well as in the high-resolution well. For 1-methyl- and 2-methylnaphthalene, which are characterized by the same physicochemical properties, at similar level of concentration a similar plume dimension was expected. Therefore, differences found may only be a result of microbial degradation. Also here 2-methylnaphthalene was indicated to be better biodegradable. Higher vertical

distribution of the dimethylnaphthalene at lower level of concentration indicated less degradability or the formation of the dimethylated compound from the parent compounds.

Meckenstock et al. (2000) were successful to isolate a naphthalene as well as 2-methylnaphthalene degrading sulfate-reducing pure culture. However, the culture was not able to grow on 1-methylnaphthalene as sole source of carbon. Cometabolic studies allowed to show the transformation of 1-methylnaphthalene to 1-methyl-naphthyl-2-methylsuccinic and 1-methyl-2-naphthoic acid as products (Safinowski et al., 2006) indicating a reaction known from the degradation of 2-methylnaphthalene. Until now, bacteria are unknown using 1-methylnaphthalene as sole source of energy and carbon.

At the Wülknitz and Düsseldorf-Flingern site 1-naphthol and 2-naphthol were present. Both compounds were found in similar range of concentration, while 1-naphthol was more widespread distributed and was still detectable downstream the plume in Wülknitz. At the Castrop-Rauxel site only 1-naphthol was determined. In the high-resolution well, the plume of 1-naphthol was found to be broader than the plume of 2-naphthol at the same level of concentration. These findings may suggest a higher transformation of 2-naphthol than 1-naphthol if they are considered as constituents of the original tar oil (see results of the analyses of different tar oils in Table 45, appendix; Eigen and Lang, 1967). Furthermore, both compounds are known as metabolites of the aerobic degradation of naphthalene.

A comparison of naphthalene with the 2-ring homocyclic compounds indan and indene shows the importance of these compounds in the groundwater of the sites. Indene was present in a similar range of concentration as has been observed for naphthalene at all the sites investigated. While similar amounts reached the testfield in Castrop-Rauxel a slower rate of degradation was seen with indene compared to naphthalene. At the other sites similar distribution and persistence of both compounds were found.

In microcosms with groundwater from the Wülknitz site, the decrease of indene was obviously less than that of naphthalene. Indene, present in lower concentration than naphthalene within the high-resolution well of Düsseldorf-Flingern, formed a plume with identical width indicating less degradability of indene. Indan was found less distributed and was decreased less over all of the field sites than naphthalene and indene. In the on-site columns of the Wülknitz site indan was eliminated in minor amounts while indene was transformed completely. Also the comparison of the plume widths in the high-resolution well indicate less degradability of indan compared to indene.

1-Indanone was found at the three sites, but it was only present in the highest contaminated regions where it reached concentrations up to the mg/L-range. Its presence may be discussed in context of microbial formation, as has been shown by Mundt et al. (2003) to occur under aerobic conditions, while its presence as constituent in tar oils has to be considered, too (see results of the analyses of different tar oils in Table 45, appendix). Independent on its origin, the results clearly show an indication for a high degradative potential towards 1-indanone, because of its high decrease with distance from the source. This was not expected from its physicochemical properties indicating high water solubility und therefore high mobility with the groundwater flow.

The 3-ring PAHs fluorene and acenaphthene exhibiting P_{OW}-values of about ~ 4 are reduced in the field sites at a lower degree than dibenzofuran. The PAHs formed broader vertical plume sizes as detected in the high-resolution well. Within these compounds acenaphthene showed highest recalcitrance. It was determined in high concentrations near the source and was found widespread over the sites leading to its presence in the wells located most downstream. These findings are in accordance to results of Zamifirescu and Grathwohl

(2001), who also classified acenaphthene to be a high recalcitrant compound at a former gasworks site.

4.2.3.2 Compounds of relevance

The results from groundwater analyses of three tar oil contaminated sites show that NSO-heterocycles were present in remarkable amounts. Although the contamination of the sites is of different origin (coking, gasification, wood impregnation) there were similar patterns of heterocycles determined at the field sites and similar characteristics in distribution were found.

Both, the data obtained here and those from the literature clearly indicate that 2- and 3-ring heterocyclic compounds are of high relevance as contaminants at those sites due to their high concentrations in tar oils and their high water solubility (Lang and Eigen, 1967). The octanol-water partition coefficients of these compounds indicate that the groundwater contained mainly compounds with log P_{OW}-values plainly less than 4.2. Theoretically, highly polar compounds are expected in tar oil contaminated groundwater, especially downstream the contamination source, because of their higher water solubility and therefore higher dissolution from the NAPL-phase. More non-polar compounds remain mostly within the tar oil phase, i. e. they are released in lower amounts into the water phase. Therefore, the remaining tar oil phase density is increasing and non-polar compounds are enriched in the source region by aging of the source. These considerations were indicated to be true at some sites described in the literature (Fowler et al., 1994; Pereira et al., 1987; Johansen et al., 1997b; Zamifirescu and Grathwohl, 2001).

A plot of decrease of contaminants over the field site in Castrop-Rauxel and Düsseldorf-Flingern as well as the decrease of compounds in the on-site columns of Wülknitz and decrease in the microcosms with groundwater of the Düsseldorf-Flingern site versus the log P_{OW} of the contaminants is shown in Figure 33. The testfield of Castrop-Rauxel with its 45 sampling points close to each other as well as the on-site columns of the Wülknitz site, which simulate the field conditions, seem to allow the best survey to observe compounds behaviour and to find differences in microbial activity. When looking at these results the presence of at least two groups concerning rate of degradation were found: (1) compounds exhibiting log P_{OW}–values higher than 3, and (2) those with log P_{OW}–values below 3. Most compounds determined in the testfield exhibiting log P_{OW}–values higher than 3 are eliminated at a lower degree, apart from some exceptions. The same is true in the on-site column experiments, where the compounds with log P_{OW}-values lower than 3 were degraded at higher rate. These findings are also indicated from the analyses of groundwater and microcosms of the Düsseldorf-Flingern site.

Compounds with log P_{OW}–values lower than 3, such as hydroxylated quinoline compounds, 1-naphthol and 1-indanone, which may be discussed in context of being degradative products, as well as benzofuran, and 1-ring-heterocyclic compounds like pyridines and thiophenes, were found in high concentrations up to the mg/L-range as it was expected considering their water solubility. However, in most cases these compounds were present only in the highest contaminated region of the sites. A high reduction in concentration of these compounds was found downstream the plume at all three sites, which often led to concentrations near or lower than the limit of detection in the downstream. The high decrease in concentration of the polar compounds in comparison to the more non-polar compounds is in contradiction to their physicochemical characteristics. An increase in concentration of the polar compounds downstream was suggested because of the higher solubility into the water phase as well as less intension of hydrophobic sorption to the aquifer material. Therefore, the

observation can only be explained by high microbial degradative activity towards these compounds.

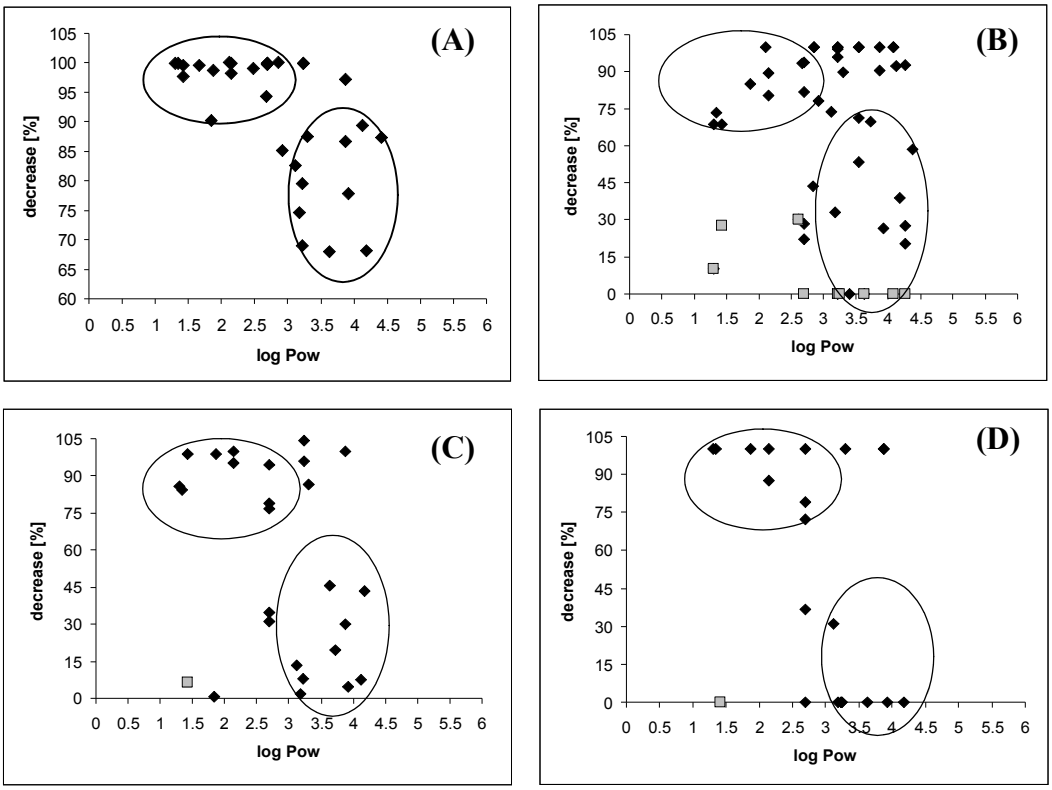


Figure 33: Correlation of decrease in concentration with log P_{OW} of compounds within (A) the groundwater of the testfield of the Castrop-Rauxel site, (B) the groundwater of the Düsseldorf-Flingern site (line 1 to line 4), (C) the on-site column of Wülknitz, and (D) the sulfate-reducing microcosms with groundwater of the Düsseldorf-Flingern site. Grey-coloured data points are assigned to compounds which may be metabolites.

The group of compounds characterized by log P_{OW} >3 has to be considered for the evaluation of groundwater downstream the source. However, the field data indicate that the group of these compounds is more heterogenic in terms of elimination in the plume. A broad number of methylsubstituted benzofurans, benzothiophenes and dibenzofurans was detected in the groundwater, especially at the Düsseldorf-Flingern site, which show a high variance in tendency of decrease. For some isomers a decrease of 100 % was found, while others showed less decrease or even an increase along the plume, which might be explained by the microbial formation from the non-substituted parent compounds.

When including these observations the characteristics of the mentioned non-polar compounds (i. e. log P_{OW} >3) have to be divided as follows:

- compounds which carry a methylsubstituent; therefore the initial microbial attack might be blocked and degradation is inhibited.
- 3-ring compounds, which may be less biodegradable because of their reduced bioavailability, e.g. dibenzofurans; reduced bioavailability might also influence the degradability of methylsubstituted compounds.
- compounds, which are highly degradable, but are still present downstream because of their high initial concentrations such as naphthalene.

- compounds, which are favoured to microbial degradation because of specialisation of microorganisms, e.g. 2-methylnaphthalene.

A combination of results from the distribution of the field and several degradation experiments is shown in Table 13. The contaminants are listed with respect of slow degradation and relevance at the field sites and therefore a necessity for an analysis in the downstream of the plume. Most compounds found downstream the source were also indicated as less biodegradable in the degradation experiments.

Many methylsubstituted compounds, parent and hydroxylated metabolites are included in the list of special attention, while the characterization of the isomers of methylsubstituted heterocycles seems to be an important task for future research.

Table 13: Evaluation of compounds relevant for groundwater analyses downstream the source.

Compounds with low degradative potential in microcosms and on-site columns	**Compounds of relevance from field data - detected downstream the source**	**Compounds of relevance for future groundwater analyses downstream a source**
N-Heterocycles		
1(2H)-isoquinolinone	1(2H)-isoquinolinone	**1(2H)-isoquinolinone**
3,4-dihydro-1(2H)-isoquinolinone	3,4-dihydro-1(2H)-isoquinolinone	**3,4-dihydro-1(2H)-isoquinolinone**
4-methyl-2(1H)-quinolinone	4-methyl-2(1H)-quinolinone	**4-methyl-2(1H)-quinolinone**
1-methyl-2(1H)-quinolinone	1-methyl-2(1H)-quinolinone	**1-methyl-2(1H)-quinolinone**
2-methylquinoline	2-methylquinoline	**2-methylquinoline**
1-methylisoquinoline		
2,4-dimethylquinoline		
2,6-dimethylquinoline		
2-methyl-4(1H)-quinolinone	2-methyl-4(1H)-quinolinone	**2-methyl-4(1H)-quinolinone**
2-methyl-6-quinolinol		
	isoquinoline	**isoquinoline**
5(6H)-phenanthridinone		
carbazole		
S-Heterocycles		
benzothiophene	benzothiophene	**benzothiophene**
	methylbenzothiophene	**methylbenzothiophene**
	dimethylbenzothiophenes	**dimethylbenzothiophenes**
O-Heterocycles		
dibenzofuran	dibenzofuran	**dibenzofuran**
	methyldibenzofurans	**methyldibenzofurans**
2-methylbenzofuran	methylbenzofurans	**methylbenzofurans**
2,3-dimethylbenzofuran	dimethylbenzofurans	**dimethylbenzofurans**
Homocycles/PAHs		
acenaphthene	acenaphthene	**acenaphthene**
fluorene	fluorene	**fluorene**
	naphthalene	**naphthalene**
1-methylnaphthalene	1-methylnaphthalene	**1-methylnaphthalene**
	2-methylnaphthalene	**2-methylnaphthalene**
	1,3-dimethylnaphthalene	**1,3-dimethylnaphthalene**
indan	indan	**indan**
indene	indene	**indene**
1-naphthol	1-naphthol	**1-naphthol**

4.3 Hydroxylated quinolines: important characteristics and fate assessment

At most contaminated sites a detailed mapping of the plume, which can demonstrate natural attenuation due to mass reduction of dissolved contaminants, i.e. the optimal appropriate condition, does not exist, a general problem for all kinds of subsurface investigations. Normally, because of financial limits largest data gaps exist about the behaviour of the source of contamination, e. g. location, distribution and mass, as well as of the hydraulic and geochemical behaviour of the aquifer. A high-resolution well system, as has been used in Düsseldorf-Flingern, is not feasible at many sites because of inaccessible high costs.

These facts indicate that there is a need for an approach, which is independent from detailed monitoring of the aquifer characteristics, to show the presence of natural attenuation easily.

Quinoline and 2(1H)-quinolinone as well as its isomeric compounds isoquinoline and 1(2H)-isoquinolinone have been detected in various aquifers of tar oil contaminated sites as well as at all three sites investigated in this study. The hydroxylated compounds 2(1H)-quinolinone and 1(2H)-isoquinolinone as well as the hydrogenated compound 3,4-dihydro-2(1H)-quinolinone are known metabolites of the anaerobic degradation of quinoline and isoquinoline. In the microcosm experiments indications were found that hydroxylation might take place at these sites.

The interpretation of the presence of hydroxylated quinolines as metabolites in groundwater is complicated, because they also may be present in the original complex mixture of tar oils as postulated elsewhere (Goerlitz et al, 1985; Johansen et al., 1997b). However, a clear proof for the occurrence of 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone and 1(2H)-isoquinolinone in tar oil is absent in the literature.

The following approaches were carried out to study, whether hydroxylated quinoline and isoquinoline can be used as indicators for natural attenuation:

- The microbial degradative potential of aquifer material towards quinoline, isoquinoline as well as methylated quinolines and metabolites under anoxic redox conditions, which are of groundwater relevance, was investigated.
- The occurrence of 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone, 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone in several tar products was examined.
- Further parameters influencing the distribution and fate of parent compounds and hydroxylated quinoline compounds were studied: these are dilution, volatilization, sorption and dissolution from tar oils.

The detailed results about the fate of these compounds at the field sites presented in chapter 4.2 are discussed in context to these data.

At first, the transformation of quinoline into hydroxylated compounds will be assessed to answer the question: Is bacterial metabolism of quinoline compounds a detoxifying process for human health and the environment?

4.3.1 Toxicological potential of quinolines and their metabolites

Most studies focussed on toxicity of high molecular weight N-heterocyclic compounds with 3-5 aromatic rings. Until now, only few studies dealt with the ecotoxicity of the 2-ring analogs quinoline and isoquinoline. Toxicity of these was reported to be lower than that of compounds with higher number of aromatic rings. There exists only scarce knowledge on toxicity of substituted quinolines such as methylsubstituted as well as hydroxylated analogs.

As has been pointed out in the precedent chapters the quinoline parent compounds were found in much lower concentrations than their hydroxylated analogs. The later reached up to the mg/L-range in the groundwater of different tar oil contaminated sites. Furthermore, the group of methylquinolines was found to be of higher relevance in the groundwater than non-substituted compounds.

On this account the environmental impact of these quinoline compounds was examined with various bioassays. The influence of substituent on quinoline compounds, furthermore the process of degradation of quinoline compounds, and formation of recalcitrant hydroxylated metabolites was assessed.

A set of the following test systems was performed to determine ecotoxicity, genotoxicity and mutagenicity:

- algae growth inhibition assay
- daphnids immobilization assay
- bacterial luminescence inhibition assay
- bacterial cell growth inhibition assay with *Pseudomonas putida* and *Vibrio fischeri*
- *umuC* test
- Ames fluctuation test

4.3.1.1 Toxic potential of quinoline compounds

The results on the ecotoxicity of a broad number of quinoline compounds are summarized in Table 14. Generally, the luminescence inhibition assay was found to be the most sensitive test system, resulting in lowest EC_{50}-values (0.1-10 mg/L) followed by the daphnids immobilization assay (8.6->208 mg/L). The algae growth inhibition and bacterial cell growth inhibition assay were less sensitive. In the growth inhibition tests *Vibrio fischeri* was found more sensitive towards the tested quinoline compounds than *Pseudomonas putida*.

Isoquinoline caused a stronger ecotoxic effect than quinoline. Ecotoxicity of these compounds was determined in the daphnids immobilization assay in a similar range, while the ecotoxic effect was about a factor 6 and 67 higher in the algae growth and the *Vibrio fischeri* luminescence inhibition assay, respectively.

The EC_{50}-values of quinoline and methylquinolines were generally found in the same range of concentration. Within the methylquinolines 6-methylquinoline was generally the most toxic one in the luminescence inhibition assay (EC_{50} = 1.4 mg/L), while 2-methylquinoline was less toxic (EC_{50} = 13 mg/L). The same tendencies were evident in the algae growth inhibition test as well as in the daphnids immobilisation assay. All methylquinolines - with the exception of 2-methylquinoline - were found to be more toxic in both assays than quinoline. While the EC_{50}-values determined for quinoline were found outside the concentration range tested, i. e. they are higher than the applied concentration, for several methylquinolines the EC_{50}-values lay within the concentration range studied. The EC_{50}-values of isoquinoline were in the same range or even lower than those of methylquinolines.

In none of the tests studied the toxicity of methylquinolines showed a correlation with a special position of a methylsubstituent in the N-heterocyclic or the homocyclic ring.

A comparison of the toxicity of quinoline and isoquinoline with its hydroxylated analogs as well as the comparison of methylquinolines with the hydroxy-methylated analogs does not show a clear trend. In the algae growth inhibition assay all compounds hydroxylated in position 2 (and for isoquinoline in position 1, respectively) are less toxic than their parent compounds. However, this trend is not obvious in the other tests performed. While sometimes the toxic effects are in a similar range or less there are even some cases where the hydroxylated compounds are found to be more toxic than their parent compounds.

Furthermore, the EC_{50}-values showed that the reduction of the hydroxylated analogs to 3,4-dihydro-2(1H)-quinolinone, 3,4-dihydro-1(2H)-isoquinolinone as well as methyl-3,4-dihydro-2(1H)-quinolinone did not result in a clear detoxification. In some cases lower toxicity was determined, while generally the EC_{50}-values of the parent compound and the metabolite were found in the same range of concentration.

Table 14: EC_{50}-values of quinoline compounds in the algae growth inhibition, the daphnids immobilization, the *Vibrio fischeri* luminescence as well as the growth inhibition assay with *Vibrio fischeri* and *Pseudomonas putida*.

compound	algae growth inhibition EC_{50} [mg/L]	daphnids immobilization EC_{50} [mg/L]	*Vibrio fischeri* luminescence inhibition EC_{50} [mg/L]	*Vibrio fischeri* growth inhibition EC_{50} [mg/L]	*Pseudomonas putida* growth inhibition EC_{50} [mg/L]
quinoline*	123.5 (88.4-172)	31.8 (21-96.7)	6.7 (6.4-7)	> 98.8	> 98.8
isoquinoline*	19.7 (16-24.5)	21.8 (12.4 - 26.3)	0.1 (0.1-0.2)	> 55.6	> 55.6
2-methylquinoline	99.2 (47.1-209.6)	65.7 (57.7-74.8)	12.8 (11.9-13.6)	> 87.6	> 108.4
3-methylquinoline	46.6 (24.2-89.8)	9.0 (4.5-17.8)	4.5 (4.3-4.7)	44.6	> 97.1
4-methylquinoline	50.8 (29.6-87.1)	25.2 (20.8-30.5)	3.8 (3.4-4.1)	51.6	42.6
6-methylquinoline	36.5 (12.8-104.4)	8.6 (4.8-15.5)	1.4 (1.3-1.6)	34.9	> 89.6
7-methylquinoline	52.5 (10.7-258.0)	14.8 (7.9-27.8)	7.3 (6.8-7.8)	38.1	47.9
8-methylquinoline	39.6 (20.9-75.0)	17.8 (15.1-21.0)	6.4 (6.1-6.8)	37.9	> 66.3
2(1H)-quinolinone*	> 176.2	63.4 (53.4-75.9)	0.9 (0.8-1)	> 110.2	> 110.2
1(2H)-isoquinolinone*	> 155.4	52.6 (44-62.8)	11.5 (10.8-12.1)	> 97.0	> 97.0
3,4-dihydro-2(1H)-quinolinone*	> 176.7	104.4 (80.1-137.6)	4.4 (4.2-4.6)	> 110.5	> 110.5
3,4-dihydro-1(2H)-isoquinolinone*	> 157.4	> 208	6.7 (6.4-7.3)	> 98.4	> 98.4
4-methyl-2(1H)-quinolinone	> 99.5	39.9 (26.5-60.3)	8.4 (7.8-9.0)	>89.7	17.6
2-methyl-6-quinolinol	44.5 (17.6-112.6)	25.1 (19.2-33.0)	11.8 (11.0-12.8)	>96.9	59.2
2-methyl-4(1H)-quinolinone	80.8 (38.9-170.1)	> 77.6	> 99.7	19.7	45.7
methyl-3,4-dihydro-2(1H)-quinolinone mixture**	79.5 (29.5-214.7)	n.d.	0.4 (0.3-0.5)	>98.9	> 105.7

*data were collected from the Diploma thesis of Neuwoehner (2006) as well as Neuwoehner et al., 2007.

** the methyl-3,4-dihydro-2(1H)-quinolinone mixture is a composition of 3 isomers (5-, 6- and 7-methylisomer, composition: 3.8:17.3:76.3 %). The quantification was performed using the sum of 6- and 7-methyl-isomer. n.d. = not determined; standard deviations of EC_{50}-values are given in brackets.

The results of the *umuC* and the Ames fluctuation test concerning genotoxicity and mutagenicity of quinoline compounds are summarized in Table 15 (information on the level of concentration are given in Table 36 and 37, appendix).

Most quinoline compounds exhibit a genotoxic and mutagenic potential after metabolic activation. All of the methylquinolines as well as quinoline itself show a mutagenic potential after the activation by S9 mix in strain TA100 (base substitution mutant), while only quinoline and 4-methyl- and 6-methylquinoline with metabolic activation were mutagenic towards strain TA98 (frame shift mutant). 7-Methylquinoline was the only isomer showing mutagenic potential without metabolic activation in strain TA100. The only hydroxylated analog, which was found to be mutagenic without metabolic activation in strain TA98 and after metabolic activation in strain TA100, was 4-methyl-2(1H)-quinolinone.

A minor portion of the investigated compounds was found to be genotoxic in the *umuC* test. These were 3-methyl- and 4-methylquinoline as well as the hydroxylated compounds 4-methyl-2(1H)-quinolinone and 2-methyl-6-quinolinol, while 4-methylquinoline, 4-methyl-2(1H)-quinolinone and 2-methyl-6-quinolinol showed genotoxicity without metabolic activation. The so-called activation resulted in a loss of genotoxicity of 2-methyl-6-quinolinol.

Table 15: Effects of quinoline compounds in the *umuC* test as well as in the Ames fluctuation test.

compound	*umuC* test		Ames fluctuation test			
			TA 98		TA 100	
	- S9	+ S9	- S9	+ S9	- S9	+ S9
quinoline*	-	-	-	+	-	+
isoquinoline*	-	-	-	-	-	-
2-methylquinoline	-	-	-	-	-	+
3-methylquinoline	-	+	-	-	-	+
4-methylquinoline	+	+	-	+	-	+
6-methylquinoline	-	-	-	+	-	+
7-methylquinoline	-	-	-	-	+	+
8-methylquinoline	-	-	-	-	-	+
2(1H)-quinolinone*	-	-	-	-	-	-
1(2H)-isoquinolinone*	-	-	-	-	-	-
3,4-dihydro-2(1H)-quinolinone*	-	-	-	-	-	-
3,4-dihydro-1(2H)-isoquinolinone*	-	-	-	-	-	-
4-methyl-2(1H)-quinolinone	+	+	+	-	-	+
2-methyl-6-quinolinol	+	-	-	-	-	-
2-methyl-4(1H)-quinolinone	-	-	-	-	-	-
methyl-3,4-dihydro-2(1H)-quinolinone mixture**	-	-	-	-	-	-

*data were taken from the Diploma thesis of Neuwoehner (2006) as well as Neuwoehner et al., 2007.
+: in the *umuC* test = genotoxic; in the Ames fluctuation test = mutagenic.
** the methyl-3,4-dihydro-2(1H)-quinolinone mixture is a composition of 3 isomers (5-, 6- and 7-methylisomer, 3.8:17.3:76.3 %). The quantification was performed using the 6- and 7-methyl-isomer.

4.3.1.2 Considerations concerning toxicity mechanisms

Different mechanisms are known which explain the toxicity of polluting compounds. A chemical may function as a baseline toxicant, a polar narcotic or reactive and specifically acting compound. Baseline toxicity describes the minimal toxicity of any pollutant and is a result of the uptake of hydrophobic pollutants into the membranes or storage lipids of an organism (Escher and Schwarzenbach, 2002). To classify the mode of toxic action of a chemical, Quantitative Structure-Activity Relationships (QSARs) are applied for the

prediction. In these correlations, the EC_{50} is related to a hydrophobicity descriptor, typically the octanol-water partition coefficient (log P_{OW}).

In several studies correlations were found between the toxicity and log P_{OW} of organic pollutants (Koenemann et al., 1981; Veith et al., 1983; De Voogt et al., 1988). Bleeker et al. (1999) found high correlations of LC_{50}-values of parent azaarenes acridine and phenanthridine and their metabolites to log P_{OW}-values in tests with the midge *Chironomus riparius*.

Here, when evaluating the EC_{50}-values from the different tests, a low correlation between EC_{50}-values of quinoline compounds in the daphnids immobilization test with log P_{OW}-values of the compounds was found (Figure 34). The linear correlation coefficient was about $R^2 = 0.5$.

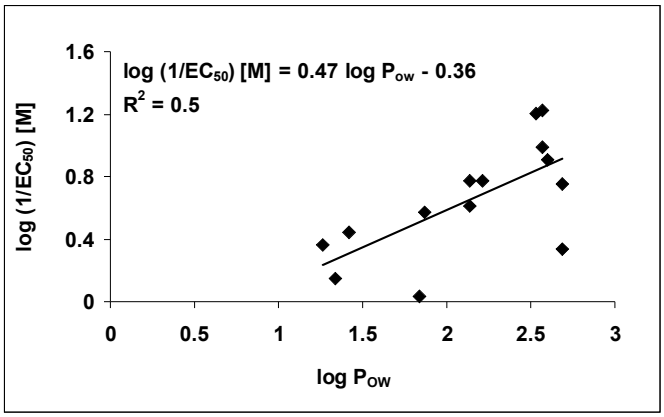


Figure 34: Correlation of log ($1/EC_{50}$) [M] of daphnids immobilization test versus log P_{OW}.

Slightly increasing toxicity with increasing log P_{OW}-value suggests that toxicity of quinoline compounds on *Daphnia magna* may be a non-specific process, where there exists a relationship between narcosis and hydrophobicity. However, the linear correlation coefficient is not as good as found in other studies and hence a specific mode of action could not be clearly dispelled for several compounds. As the pH of the test system was neutral, the charged form of several methylquinolines (pKa-values of 5.2-5.9) may play a role in the mechanism of toxicity, while for all other quinoline compounds the charged species may not be of importance because of pKa-values more than two units lower than pH 7.

Furthermore, it has to be considered that the range of log P_{OW}-values of compounds investigated here was plainly smaller, resulting in a higher deviation from the linearity compared to studies mentioned above. Anyhow, a further evaluation of the mode of action was performed. Two approved QSAR-models were used to evaluate the experimental data. A detailed comparison of every experimental EC_{50}-value with predicted values was performed. At first the equation of Zhao et al. (1998) (log ($1/EC_{50}$(M)) = 0.81 log P_{OW} + 1.64) was used, which is a result of correlation of EC_{50}-values of 87 compounds with log P_{OW}-values in the range of 1-5. Furthermore, a comparison of experimental with predicted data from the ECOSAR-Program (U.S. EPA, 2000), also using the octanol-water partition coefficient as basis, was done. These calculated EC_{50}-values are given in Table 16. To assess the results, the experimental EC_{50}-values were compared to calculated EC_{50}-values and evaluated according to the approach described by Verhaar et al. (1992) using the toxic ratio (TR):

$$TR = \frac{EC_{50(predicted)}}{EC_{50(calculated)}}$$

These authors classified a chemical as baseline toxicant if TR < 5, as polar narcotic if TR ≥ 5 and ≤ 10, and as a reactive or specifically acting compound if TR ≥ 10.

At a first glance both, the equation obtained from Figure 34 as well as that described by Zhao et al. (1998), indicated baseline toxicity in the *Daphnia magna* test, which was confirmed by the comparison of each single experimental and predicted value (Table 16). Predicted EC_{50}-values and experimental ones were in good accordance, while a highest deviation for 2(1H)-quinolinone and 1(2H)-isoquinolinone was found, which identified these compounds as polar narcotics.

Table 16: Comparison of predicted and experimental EC_{50}-values for *Daphnia magna* and *Vibrio fischeri* luminescence inhibition assay.

compound	log P_{OW}	V_x	***Daphnia magna* assay**					***Vibrio fischeri* luminescence inhibition assay**		
			EC_{50} (exp) [mg/L]	EC_{50} (pred) (1) [mg/L]	TR (1)	EC_{50} (pred) (2) [mg/L]	TR (2)	EC_{50} (exp) [mg/L]	EC_{50} (pred) [mg/L]	TR
quinoline	2.14	111.0	31.8	76.5	2.4	54.3	1.7	6.7	50.5	7.5
isoquinoline	2.14	111.0	21.8	76.5	3.5	54.3	2.5	0.1	19.2	192
2-methylquinoline	2.69	125.1	65.7	26.8	0.4	21.8	0.3	12.8	6.6	0.5
3-methylquinoline	2.69	125.1	9.0	26.8	3.0	21.8	2.4	4.5	6.6	1.5
4-methylquinoline	2.69	125.1	25.2	26.8	1.1	21.8	0.9	3.8	6.6	1.5
6-methylquinoline	2.69	125.1	8.6	26.8	3.1	21.8	2.5	1.4	6.6	4.7
7-methylquinoline	2.69	125.1	14.8	26.8	1.8	21.8	1.5	7.3	6.6	0.9
8-methylquinoline	2.69	125.1	17.8	26.8	1.5	21.8	1.2	6.4	6.6	1.0
2(1H)-quinolinone	1.26	116.9	63.4	n.a.	n.a.	317	5	0.9	45.0	50
1(2H)-isoquinolinone	1.42	116.9	52.6	388.7	7.4	235	4.5	11.5	37.7	3.3
3,4-dihydro-2(1H)-quinolinone	1.34	121.2	104.4	313.0	3.0	277	2.6	4.4	37.4	8.5
3,4-dihydro-1(2H)-isoquinolinone	1.34	121.2	>208	248.6	<1.2	277	>1.3	6.7	37.4	5.6
4-methyl-2(1H)-quinolinone	1.87	131.0	39.9	n.a.	n.a	112	2.8	8.4	4.8	0.6
2-methyl-6-quinolinol	2.21	131.0	25.1	7.03	0.3	59.2	2.4	11.8	9.8	0.8
2-methyl-4(1H)-quinolinone	1.84	131.0	>77.6	87.2	<1.1	118	1.5	>99.7	14.3	<0.1
methyl-3,4-dihydro-2(1H)-quinolinone mixture	2.08	135.3	n.d.	108.3	n.a	75.8	n.a.	0.4	102.4	255

The McGowan characteristic volume (V_x) used for the correlation of log P_{OW}-value and results from the *Vibrio fischeri* luminescence inhibition assay according to Zhao et al. (1990) was calculated by the summation of the following atomic and bond contributions: C 16.35; H 8.71; O 12.43; N 14.39. For each bond the factor 6.56 was subtracted. n.d. = not determined; n.a. = not available.
Predicted data: (1) according to U.S. EPA (2000) and (2) according to Zhao et al. (1998).

Neither the algae growth nor the *Vibrio fischeri* luminescence inhibition assay, showed a linear correlation of EC_{50}- and log P_{OW}-values. But only a limited number of data was available because of the EC_{50}-values which were found higher than those concentrations studied in the tests. Van Vlaardingen et al. (1996) failed to find a relationship between toxicity of azaarenes and log P_{OW} in the algae test with *Scenedesmus acuminatus*, but they obtained tentative indications that electronic interactions play a role in differences in toxic actions. The QSAR prediction by the ECOSAR program (U.S. EPA, 2000) leads to EC_{50}-values for green algae (data not shown), which are mostly 2-6-fold lower than those found in algae test with *Desmodesmus subspicatus*. Therefore, the presence of a specific action of the chemicals is not indicated here.

Zhao et al. (1998) found a correlation for EC_{50}-values and log P_{OW} within the *Vibrio fischeri* luminescence inhibition assay. However, the correlation could be improved by including the additional model descriptor, the McGowan characteristic volume (V_x) into the equation, which shows the important role of molecular bulk. In contrast to *Daphnia magna*, the lipid content of the unicellular organism *Vibrio fischeri* is low, and hence organic chemical accumulation was not expected to greatly increase with compounds hydrophobicity (increasing log P_{OW}). Therefore, Zhao et al. (1998) found the highest linear correlation using the following equation: $\log (1/EC_{50})\ (M) = 0.475 \log P_{OW} + 4.99 \log V_x - 7.42$.

The last mentioned equation was used for the calculation of EC_{50}-values of quinoline compounds. In addition, experimental data were included in a comparison to assess the toxic ratio as described above. As presented in Table 16 for all methylquinolines and hydroxyl-methylated quinolines, baseline toxicity is indicated in *Vibrio fischeri* luminescence inhibition assay. In contrast, for quinoline, 3,4-dihydro-2(1H)-quinolinone, 3,4-dihydro-1(2H)-isoquinolinone polar narcosis is demonstrated by a toxic ratio higher than 5, while even specific mechanisms of toxicity are clearly shown for 2(1H)-quinolinone, isoquinoline and the methyl-3,4-dihydro-2(1H)-quinolinone isomer mixture, with toxic ratio >>10.

4.3.1.3 Assessment of toxicity of tar oil contaminated groundwater

The EC_{50}-values of quinoline compounds have to be discussed in context of occurrence and level of concentration of quinoline compounds in groundwater of tar oil contaminated sites.

Regarding the toxic potential, isoquinoline was found to be more toxic than quinoline in all test systems investigated. Even if there is no genotoxic and mutagenic potential of isoquinoline, ecotoxic effects are of high relevance for tar oil contaminated groundwater, since isoquinoline is often found downstream a contamination plume in contrast to quinoline, which is mostly detectable in low concentrations near the source of contamination (chapter 4.2.1).

The data on toxicity show that a clear assessment of the influence of biodegradative processes on the toxic potential of a quinoline compound is not possible. Since the hydroxylation of quinoline and isoquinoline leads to more hydrophilic compounds lower toxicity was expected for hydroxylated compounds in comparison to the parent compounds. This tendency was seen in the daphnids immobilisation and in the algae growth inhibition assay, while in the *Vibrio fischeri* luminescence inhibition assay a clear trend is absent: both higher as well as lower toxicity of hydroxylated quinolines was indicated compared to the parent compounds. Concerning genotoxicity and mutagenicity, the hydroxylation is clearly a positive step in detoxification: none of the hydroxylated compounds 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone, 1(2H)-isoquinolinone and 3,4-dihydro-2(1H)-isoquinolinone showed an adverse effect in the *umuC* or Ames fluctuation test.

A comparison of quinoline with different methylquinolines clearly indicated that in all test systems used methylquinolines (or at least one isomer) were found to be more ecotoxic than quinoline itself. As the methylation leads to compounds with log P_{OW}-values about 0.6 units higher than the parent compound quinoline, a higher toxicity was expected from their physicochemical characteristics. Since methylsubstituted quinolines are present more widespread and in higher concentrations as well as detectable even downstream the contamination source at the three tar oil contaminated sites investigated here, the higher toxicity is an important aspect for the assessment of groundwater quality.

The assessment of the ecotoxic effects of the methylsubstituted parent compound and the hydroxylated metabolite showed no clear trend. In the Ames test all methylquinolines were

found to be mutagenic with metabolic activation, while genotoxicity in the *umuC* test was only found for the 3-methyl- and 4-methyl-isomer. As mentioned above, the microbial degradation of quinoline compounds leads to a detoxification and therefore, diminishes the risk potential for non-substituted quinoline. However, this tendency is not true for methylquinolines: 4-Methyl-2(1H)-quinolinone was found to be genotoxic with and without metabolic activation in the *umuC* test and also mutagenic in the Ames fluctuation test with metabolic activation. Therefore, a microbial degradation of 4-methylquinoline leading to the accumulation of 4-methyl-2(1H)-quinoline does not represent a detoxification mechanism. These results clearly show the importance to evaluate the toxicity of additional hydroxy-methylated quinoline compounds, which are known as metabolites and are present in high concentrations (up to the mg/L-range) in the groundwater of tar oil contaminated sites, but could not be studied until yet because of the absence of commercial available standard compounds.

When comparing EC_{50}-values obtained for quinoline compounds with concentrations present in groundwater of tar oil contaminated sites (chapter 4.2.1) a direct adverse effect of groundwater may be seen in the *V. fischeri* luminescence inhibition assay. Comparing EC_{50}-values found in *Daphnia magna* assay with concentrations in the groundwater of the three sites, the EC_{50}-values for most quinoline compounds are higher than concentrations present in the groundwater. Especially for the hydroxylated quinolines like 2(1H)-quinolinone, 1(2H)-isoquinolinone, 3,4-dihydro-2(1H)-quinolinone and 4-methyl-2(1H)-quinolinone, which are present in highest concentrations, even in the mg/L-range at the Castrop-Rauxel site, the concentrations in the groundwater are only about a factor 10-50 lower than the EC_{50}-values found. In the algae growth inhibition as well as the bacteria growth inhibition assay, the quinoline compounds with lowest EC_{50}-values are methylsubstituted quinolines. At least in the highest contaminated groundwater sample 4-methylquinoline was present in a concentration of a factor ~85 lower than its EC_{50}-value.

Since tar oil contaminated groundwater contains a complex mixture of compounds, toxic effects of mixtures have to be evaluated. For those compounds that share a common mode of toxic action, as clearly found for several quinoline compounds in *Daphnia magna* assay, cumulative exposure must be considered because such compounds typically act in additive manner. The combined toxicity of mixtures of chemicals (for the group of investigated quinoline compounds) was calculated according to:

$$EC_{y_{mix}} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{y_i}} \right)^{-1}$$

$EC_{y\,mix}$ is the concentration of the mixture that induces an overall effect y (here y = 50). EC_{yi} is the concentration of the *i*th component in an n-component mixture required inducing the same magnitude of effect y. p_i is the proportion of the *i*th component in the mixture (Backhaus et al., 2000; Escher et al., 2005).

The calculation of EC_{50mix}-values showed that even for highest contaminated groundwater samples of the Castrop-Rauxel, the Wülknitz and Düsseldorf-Flingern site, a direct toxic effect towards *Daphnia magna* caused by the only presence of investigated quinoline compounds cannot be expected, because EC_{50}-values of the groundwater samples were found within ~50 mg/L. However, the sum of quinolines in groundwater samples of the Castrop-Rauxel and Wülknitz site are within a factor of 5-15 below the threshold concentration. It has to be kept in mind that only a restricted number of compounds within a broad number of heterocyclic compounds, PAHs and further unknown contaminants was included for the calculations. Therefore, the testing of real groundwater samples downstream the plume is a

demanding task to evaluate its toxic potential and quality of groundwater besides analytical strategies.

However, for a risk assessment a special safety factor has to be included. For compounds where only toxicity data of short-term studies exist (like the presented results) normally a factor of 1000 is included to define the Predicted no Effect Concentration (PNEC). Below this concentration a negative effect of a chemical for the environment is not expected. Hence, it is obvious that a broad number of groundwater samples considerably exceeds this threshold, when only evaluating the group of quinolines. Even in groundwater samples of the second line of the testfield in Castrop-Rauxel and 100 m distance from the source of the Wülknitz site, PNEC is exceeded for hydroxylated quinoline compounds (especially 1(2H)-isoquinolinone and 4-methyl-2(1H)-quinolinone). Further downgradient the concentration in groundwater samples are lower than PNEC at all of the sites.

4.3.2 Parameters influencing quinoline compounds in groundwater

4.3.2.1 Anaerobic degradation of quinoline compounds

In the following chapter the degradation of quinoline, isoquinoline as well as methyl-, hydroxy- and hydroxy-methylsubstituted quinolines under nitrate-, sulfate- and iron-reducing conditions in microcosms with aquifer material of the former coke-manufacturing site in Castrop-Rauxel is reported.

Published data on the degradation of quinolines and isoquinolines resulted from studies with pure or mixed cultures from different origins. The relevance of the described degradative pathways (Figure 4) at contaminated sites, however, and the occurrence of parent compounds and their metabolites at such sites has to be evaluated.

Transformation of quinoline, isoquinoline and its methylsubstituted, hydroxylated and reduced analogs under anaerobic conditions was investigated over a period of at least 300 days using aquifer material of the tar oil contaminated site of Castrop-Rauxel as inoculum. An overview of the results of all degradation experiments is presented in Table 39. An insignificant abiotic degradation or sorption of compounds was seen in the control microcosms.

Theoretical consideration on microbial degradability

Thermodynamics can be used as a tool to predict whether an organism can in principle obtain energy for growth by catalyzing a reaction (Thauer et al., 1977). The calculation of changes in Gibbs free energy values are a useful tool to evaluate whether a reaction is an exergonic or endergonic process. These calculations were done for all quinoline compounds studied.

Results showed that the anaerobic microbial mineralisation of quinoline compounds under sulfate-, iron- and nitrate-reducing conditions is an exergonic process shown by the theoretical energy conservations (complete set of data is presented in chapter 7.6.2, appendix). For an adequate comparison of energetics of the different redox conditions $\Delta G^{o'}_{(aq)}$-values have to be related to the same number of electrons transferred. The $\Delta G^{o'}_{(aq)}$-values of nitrate- and iron-reduction have to be multiplied by a factor of 1.6 and 8, respectively, to allow a relation to sulfate-reducing conditions.

In general, nitrate-reducing conditions are thermodynamically more favourable than iron-reduction, and iron-reduction is more favourable than sulfate-reduction.

A comparison of $\Delta G^{o'}_{(aq)}$-values of quinoline and 2(1H)-quinolinone (and its isomeric compounds, respectively) showed that the degradation of hydroxylated analogs leads to the formation of higher energy. These calculations suggest that the step of hydroxylation of quinolines requires energy.

When estimating and predicting the rate of mineralisation of quinoline compounds *in situ*, several other factors may influence the rate of degradation such as availability of heterocyclic compounds via diffusion, kinetics of the initial activating reaction, substrate specificity of the degradative enzymes, efficiency of subsequent reactions leading to CO_2, abundance of relevant microorganisms. However, the calculation of Gibbs free energies of formation show that degradation of quinoline compounds may generally be expected under environmental conditions.

Transformation of quinoline, formation and transformation of metabolites

The rates of transformation of quinoline as well as formation and further transformation of 2(1H)-quinolinone varied under the three redox conditions tested. Fast decrease of concentration of quinoline was found under all redox conditions.

When searching for metabolites, stoichiometric amounts of the first known metabolite, 2(1H)-quinolinone, were produced within 20 days under sulfate- and iron-reducing conditions (Figure 35 A). Contrarily, an accumulation of a metabolite was absent in nitrate-reducing microcosms, even though quinoline disappeared. Even supplementing a second portion of quinoline did not lead to a detectable concentration of 2(1H)-quinolinone (< 0.003 µM) (Figure 35 A). When comparing the lag phases (Table 39) and the transformation rates of quinoline and 2(1H)-quinolinone as single compounds in nitrate-reducing microcosms, a shorter lag phase and a significantly higher transformation rate of the metabolite was seen (Figure 35 A and B). This might explain why transient accumulation of 2(1H)-quinolinone in the quinoline-supplemented microcosms was not found.

In contrast to nitrate-reducing conditions, under sulfate- and iron-reducing conditions the initial hydroxylating step seems to be faster than the following one (Table 39 and Figure 35 B). Under these conditions, further transformation of 2(1H)-quinolinone seems to be a bottleneck, leading to the accumulation of 2(1H)-quinolinone. However, further transformation of the accumulated 2(1H)-quinolinone took place under all conditions.

In sulfate- and iron-reducing microcosms with quinoline or 2(1H)-quinolinone as single substrate, 3,4-dihydro-2(1H)-quinolinone was detected in low concentrations up to an amount of 5 µM, which is about 6 % of the quinoline added. Besides the comparison with standard compound, the identification of the metabolite was performed by high-resolution mass spectrometry. The composition was predicted as $C_9H_{10}O_1N_1$ (mass of parent ion: 148.0757) with an error of 0.33 ppm (Table 41 and Figure 59). A comparison of the MS-spectrum measured and a simulated one of the proposed compound showed an exact match, including isotopic pattern of $^{12}C/^{13}C$. These data support the identification of 3,4-dihydro-2(1H)-quinolinone as metabolite.

Under nitrate-reducing conditions, degradation of quinoline was not accompanied by the formation of 3,4-dihydro-2(1H)-quinolinone, while minor amounts of 3,4-dihydro-2(1H)-quinolinone (2 µM) occurred during the degradation of 2(1H)-quinolinone (Figure 35 B).

When studying the degradation of 3,4-dihydro-2(1H)-quinolinone itself, a fast transformation without significant lag phase was observed, when the metabolite was added as sole substrate under all three redox conditions tested (Table 39 and Figure 35 C). This indicates a presence or fast induction of the degradative activity of the microcosm population.

A decrease of the electron acceptor sulfate or nitrate in microcosms with quinoline, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone as substrate was observed. In case of iron-reducing conditions, the concentration of dissolved iron increased. The concentration of the electron acceptor remained constant in the biotic controls, when only soil and medium without any substrate were given. As long as the hydroxylation took place, the concentration of the electron acceptors remained constant and changed when degradation of 2(1H)-quinolinone started. This indicates that the observed consumption of electron acceptor is only due to the mineralization of hydroxylated quinoline derivatives. A correlation of the amounts of quinoline compound (quinoline, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone) and sulfate or nitrate used as endacceptor, allowed to explain a complete mineralization of these compounds (data see Table 40, appendix), while a correlation with dissolved iron allows to explain minor mineralization.

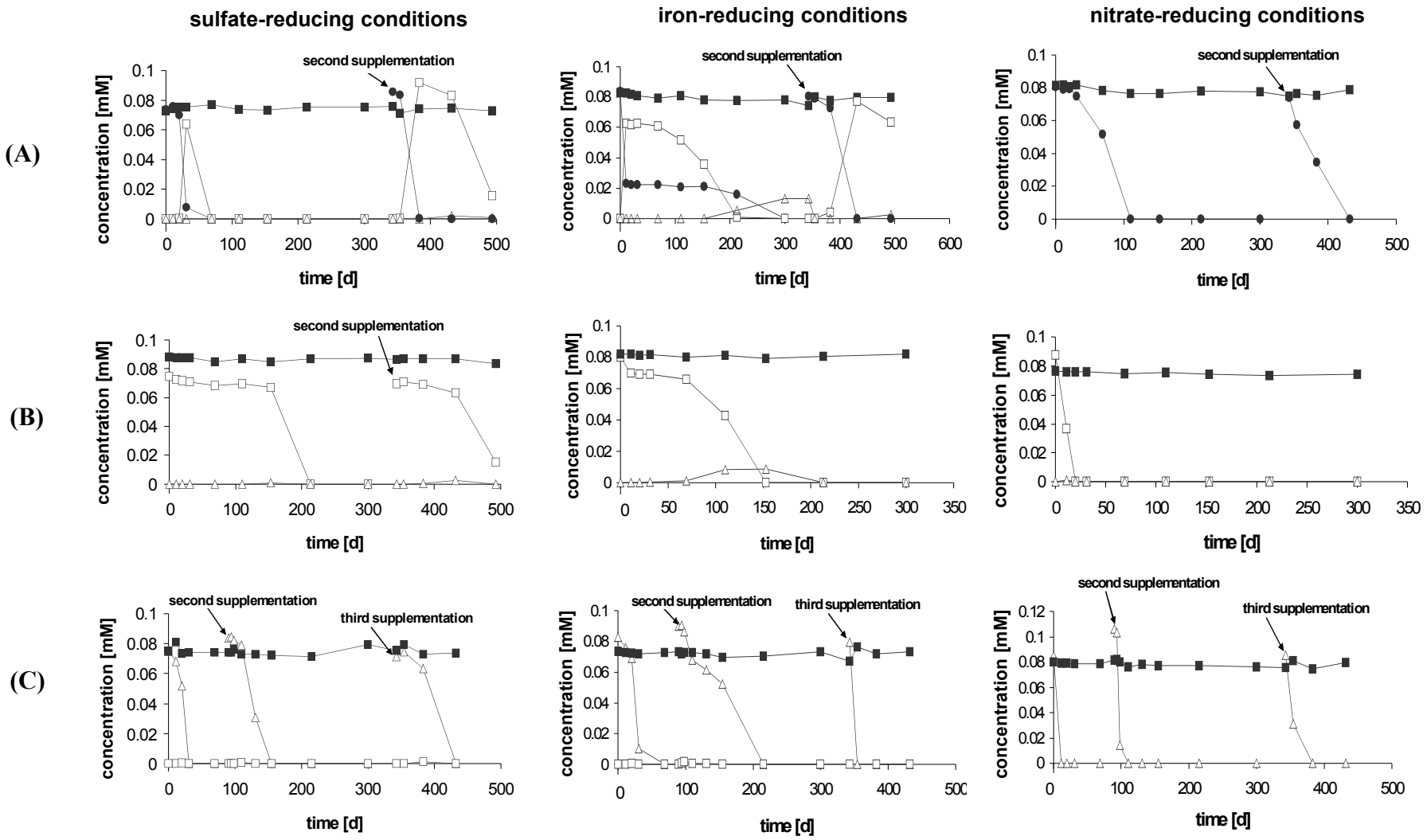


Figure 35: Transformation in microcosms supplemented with (A) quinoline, (B) 2(1H)-quinolinone and (C) 3,4-dihydro-2(1H)-quinolinone under different redox-conditions. Arrows mark a second/third supplementation of the substrate. Legend: quinoline (•), 2(1H)-quinolinone (□), 3,4-dihydro-2(1H)-quinolinone (△), abiotic control (■).

Transformation of isoquinoline, formation and transformation of metabolites

Isoquinoline showed a different transformation behaviour compared to degradation of quinoline. 1(2H)-Isoquinolinone was observed as degradation product under all three redox conditions tested, but the rates and degree of transformation varied. In contrast to the fast and complete transformation of quinoline leading to its hydroxylated product, hydroxylation of isoquinoline was obviously slower in sulfate-reducing microcosms. Less than 50 % of isoquinoline transformed was found as 1(2H)-isoquinolinone, which was further degraded after 150 days (Figure 36 A). When 1(2H)-isoquinoline was tested as substrate, high persistence was observed. Only after an incubation period of more than 200 days a noticeable decrease was found (Figure 36 B), which is in correlation with the starting decrease of sulfate. The amount of isoquinoline degraded may explain 100 % of mineralization in sulfate-reducing microcosms, if the amount of 2(1H)-isoquinolinone accumulated was considered.

In the iron-reducing microcosms concentrations of both isoquinoline and 1(2H)-isoquinolinone were constant within an incubation period of 300 days. Only small amounts (up to 2 µM = 3 %) of isoquinoline were transformed to 1(2H)-isoquinoline, which accumulated while no iron-reduction occurred (Figure 56).

In nitrate-reducing microcosms hydroxylation of isoquinoline started fast and high amounts of 1(2H)-isoquinolinone accumulated (62 µM = 77 %). However, a stoichiometry of consumed parent compound and accumulated metabolite was absent. The concentration of 1(2H)-isoquinolinone, when tested as the sole substrate, remained constant over the whole experimental period of time (Figure 56). As found in the sulfate-reducing microcosms also in the nitrate-reducing microcosms, the decrease of nitrate may explain a complete mineralisation of isoquinoline, when considering the accumulated metabolite.

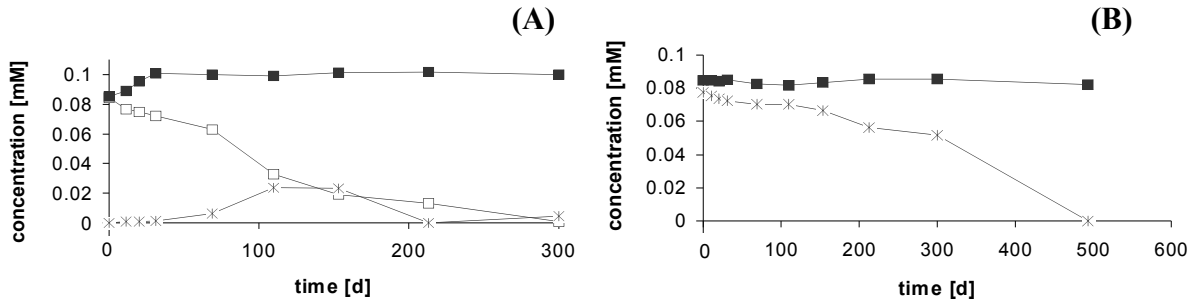


Figure 36: Transformation of (A) isoquinoline and (B) 1(2H)-isoquinolinone under sulfate-reducing conditions in microcosms with aquifer material. Isoquinoline (□), 1(2H)-isoquinolinone (✕), abiotic control (■).

Transformation of methylquinolines, formation and transformation of metabolites

Transformation of 2-methyl-, 2,4-dimethyl- and 2,6-dimethylquinoline as well as 2-methyl-4(1H)-quinolinone was absent in all microcosms studied here, independent on the redox conditions tested.

3-Methyl- and 4-methylquinoline, in contrast to 2-methylquinolines, were hydroxylated under sulfate-reducing conditions (Figure 37). In microcosms containing iron or nitrate as electron acceptor, both chemicals persisted over the investigated period of 300 days. The sulfate-reducing microbial population was able to transform 3-methylquinoline completely within 70 days. NMR-analyses of the transformation product of 3-methylquinoline showed that the hydroxylation took place in position 2 (Table 29, appendix). 3-Methyl-2(1H)-quinolinone,

quantified by using 4-methyl-2(1H)-quinolinone as standard compound, was formed in stoichiometric amounts, but persisted further transformation.

Production of 4-methyl-2(1H)-quinolinone from 4-methylquinoline in sulfate-reducing microcosms was found within 69 days (Figure 37), followed by a decrease in concentration of the transformation product with accompanied reduction of sulfate, which may explain a degradation of 80 % (Table 40, appendix). A second supplementation resulted in a faster degradation, showing an adaptation of microorganisms. When testing 4-methyl-2(1H)-quinolinone as sole substrate, degradation took place after a lag phase of 150 days. An additional portion of 4-methyl-2(1H)-quinolinone was degraded indicating again an adaptation to the substrate. A second metabolite formed in small amounts (~3 µM) was detected. Since the low concentration did not allow NMR measurements the elemental composition was determined by high-resolution mass spectrometry. The composition was predicted as $C_{10}H_{12}O_1N_1$ (mass of precursor ion of 162.0912 with an error of 0.62 ppm). The predicted mass with the isotopic pattern of $^{12}C/^{13}C$, in addition, is coincident to protonated 4-methyl-3,4-dihydro-2(1H)-quinolinone, which is the metabolite with high probability (Table 41 and Figure 60, appendix). A degradation of 4-methyl-2(1H)-quinolinone under iron-reducing conditions was also observed, whereas this compound was found to persist any transformation in the nitrate-reducing microcosms (Figure 57, appendix).

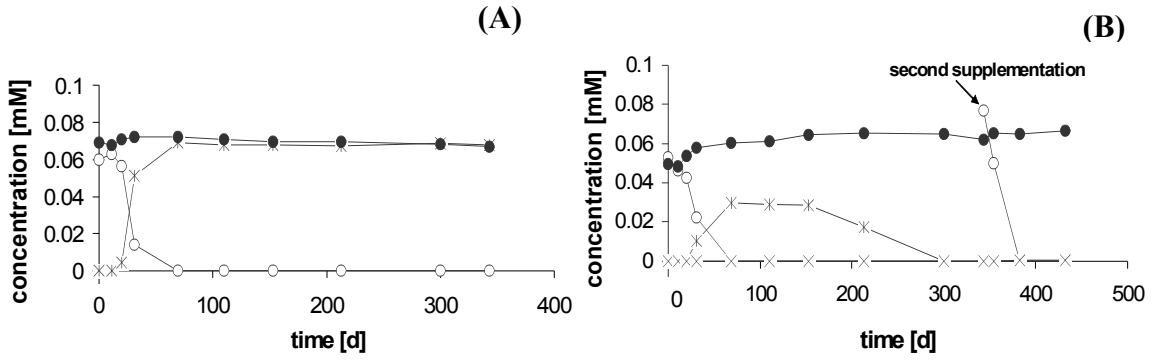


Figure 37: Transformation of (A) 3-methyl- and (B) 4-methylquinoline under sulfate-reducing conditions in microcosms with aquifer material. The arrow marks a second supplementation of the substrate. 3-Methyl- or 4-methylquinoline (○), 3-methyl- or 4-methyl-2(1H)-quinolinone (✳), 4-methyl-3,4-dihydro-2(1H)-quinolinone (×), abiotic control (•).

Quinolines substituted in the homocycle (6-, 7- and 8-methylquinoline) were also found to be transformed to the corresponding methyl-2(1H)-quinolinone under sulfate-reducing conditions. Stoichiometric amounts of the respective methyl-2(1H)-quinolinone were formed from 6-methyl- and 8-methylquinoline after an incubation period of 70 days. The hydroxylation of 7-methylquinoline led to stoichiometric amounts of 7-methyl-2(1H)-quinolinone, which reached a maximum after an incubation time of 200 days. Relevant further transformation of the 6-methyl-, 7-methyl- and 8-methylquinoline derivatives was absent within the incubation time of more than 300 days (Figure 58). Only minor amounts of the reduced metabolites 6-methyl- and 7-methyl-3,4-dihydro-2(1H)-quinolinone (0.7-5 µM = 0.9-6 %) were identified using high-resolution mass spectrometry (mass of precursor ion 162.0914/ 162.0913 was predicted as $C_{10}H_{12}O_1N_1$ with an mass error of 0.62/0.31 ppm) (Figure 61 and 62, appendix).

Under iron-reducing and nitrate-reducing conditions methylquinolines substituted in the homocyclic ring were transformed only in minor amounts. 0.1 to 10 µM in maximum of methyl-2(1H)-quinolinone were formed and no other metabolites were detected (Figure 58).

Transformation of further hydroxylated quinoline compounds

The degradation of 2(1H)-quinolinone and 1(2H)-isoquinolinone has been discussed in context to the parent compounds. Also for further hydroxy-analogs such as 4(1H)-quinolinone, 3(2H)-isoquinolinone and 5- and 7-isoquinolinol highest microbial degradative activity was found under sulfate-reducing conditions. Under these conditions all hydroxylated quinoline compounds analysed were transformed within the experimental time. The results showed that independent on the substituent hydroxylation took place in position 2 for quinoline and position 1 for isoquinoline compounds.

In iron- as well as in nitrate-reducing microcosms transformation of 3(2H)-isoquinolinone and 4(1H)-quinolinone was detected. 5-Isoquinolinol was only transformed after 300 days. 7-Isoquinolinol was not transformed within the experimental time.

In all microcosms, where a transformation took place, hydroxylated quinolines were converted into the respective dihydroxy-analogs. These metabolites were characterized by high-resolution mass spectrometry as $C_9H_8O_2N_1$ (for mass of precursor ions and analytical error see Table 41; Figure 64 and 65). The metabolite of 7-isoquinolinol was further characterized by ^{1}H-NMR showing the second hydroxylation in position 1 (A. Preiß, Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany; personal communication).

The amount of dihydroxylated metabolite formed could only be quantified for 4-hydroxy-2(1H)-quinolinone because of the absence of standards. The results show that 4-hydroxy-2(1H)-quinolinone was formed in stoichiometric amounts in the meantime. Generally, the other dihydroxyquinoline metabolites showed high persistence, while only in case of 4-hydroxy-2(1H)-quinolinone a further transformation was observed (Figure 38).

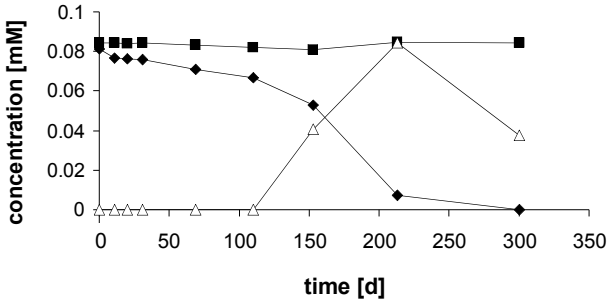


Figure 38: Degradation of 4(1H)-quinolinone under sulfate-reducing conditions; 4(1H)-quinolinone (◆), 4-hydroxy-2(1H)-quinolinone (△), abiotic control (■).

Discussion and conclusion on microbial transformation in microcosms

A comparison of degradation potential and rate under different redox conditions revealed highest degradative activities under sulfate-reducing conditions, although the higher redox potential of nitrate- and iron-reducing conditions was expected to be energetically favoured. This indicated an adaptation of microorganisms to the prevailing sulfate-reducing field conditions as described elsewhere (Licht et al., 1996).

The initial transformation for all quinoline compounds proceeded via a hydroxylation in position 2 and, respectively, in position 1 for isoquinoline compounds. This initial step has been reported under various redox conditions (Figure 4; Johansen et al, 1997a,c; Pereira et al., 1987; Liu et al., 1996). For quinoline a hydroxylation in position 2 was found under sulfate- as well as iron-reducing conditions. However, no hydroxylated metabolite was detected in nitrate-reducing microcosms, probably as a result of differences in transformation rates of

parent and hydroxylated compound. This observation was also described by Liu et al. (1994a).

The formed metabolite 2(1H)-quinolinone accumulated up to stoichiometric amounts in sulfate- and iron-reducing microcosms, while the further degradation was found as to be a slow step. Further degradation of the accumulated 2(1H)-quinolinone was also shown in other studies (Johansen et al., 1997a,c; Liu and Kuo, 1996; Licht et al., 1997a; Godsy et al., 1992; Battersby and Wilson, 1989), whereas Liu et al. (1994a) and Pereira et al. (1987 and 1988) did not observe further degradation of 2(1H)-quinolinone. However, minor knowledge exists about the further degradative pathway. Here we detected the hydroxylated and hydrogenated compound 3,4-dihydro-2(1H)-quinolinone as a second metabolite in sulfate- and iron-reducing microcosms. Until now the formation of this compound was only described in the pure culture of *D. indolicum* under sulfate-reducing conditions as well as by a mixed population from waste water treatment plant under denitrifying conditions (Johansen et al., 1997 a,c).

The much lower transformation rate of isoquinoline into its hydroxylated metabolite in sulfate- and iron-reducing microcosms compared to that of quinoline is in agreement with data obtained with mixed populations from the environment (Pereira et al., 1987; Johansen et al., 1997a). The accumulation of 1(2H)-isoquinolinone as found here has not yet been described under sulfate- and iron-reducing conditions.

Quinolines carrying a methylsubstituent in position 2 are known to persist under anoxic conditions (Shanker and Bollag, 1990; Johansen et al., 1997a,c; Pereira et al., 1987; Liu et al., 1994b). Similarly, 2-methyl- as well as 2,4- and 2,6-dimethylquinoline persisted in all microcosms tested. This result confirms the assumption that the initial attack takes place only in position 2, which is blocked by a methylsubstituent in the above tested quinoline analogs.

All other methylquinoline compounds investigated (3-, 4-, 6-, 7-, 8-methyl-) were hydroxylated in position 2. The hydroxylated metabolites generally showed high recalcitrance and were only rarely degraded further within the period of about 300 days. The reduced species 4-methyl-, 6-methyl-, and 7-methyl-3,4-dihydro-2(1H)-quinolinone were identified by high-resolution LC-MS as the further metabolites. Also Johansen et al. (1997a,c) observed the formation of these hydrogenated methylquinolines, i.e. the production of 6-methyl- and 8-methyl-3,4-dihydro-2(1H)-quinolinone, while the described formation of 4-methyl-3,4-dihydro-2(1H)-quinolinone was not observed until now.

The high persistence of methylsubstituted quinoline metabolites is in accordance to findings obtained with mixed cultures under nitrate-reducing and methanogenic conditions as well as a pure culture under sulfate-reducing conditions (Johansen et al., 1997a,c; Liu et al., 1994b). Further transformation of the metabolites of quinoline compounds substituted in position 4 (4-methylquinoline and 4(1H)-quinolinone) is determined here for the first time.

Concluding, the results show that the initial hydroxylation of quinoline compounds is present under a wide variety of redox conditions. Therefore, the presence of the degradative pathway found in pure bacterial culture and in denitrifying mixed culture (Johansen et al. 1997a,c) seems to be also of importance in the aquifer of a tar oil contaminated site.

Until yet, pure organisms or mixed cultures able to use methylquinolines as sole source of carbon under anoxic conditions have not been reported. This indicates a persistence of hydroxylated metabolites and might explain the high amounts of methylsubstituted quinolines in groundwater of tar oil contaminated sites.

4.3.2.2 Sorption of quinoline compounds

Sorption leads to a retardation of a contaminant in the ground relative to the average seepage velocity of groundwater and to a reduction of concentration in the water phase. Physical, chemical and electrostatic forces as well as coordination bonding may be involved in the process. Contaminant retardation is strongly influenced by sorption to organic and mineral solids. The nature and extent of sorption also influences the availability of contaminants for biodegradation. The structural and physicochemical properties of the contaminant as well as the physicochemical properties of the sorbent play an important role for the nature and extent of sorption (Schwarzenbach et al., 2003).

The aim of this chapter is to investigate sorption behaviour of different quinoline compounds (parent and hydroxylated quinoline compounds) on the clay mineral montmorillonite as well as on aquifer material of the Castrop-Rauxel site. The results should lead to an understanding of differences in sorption of parent compounds and metabolites and thus retardation in the aquifer.

Isotherms

Studies on kinetics of the sorption process showed that within a few minutes equilibrium was reached for all quinoline compounds on aquifer material as well as on montmorillonite. For 2(1H)-quinolinone equilibrium was achieved within 10 min, while it took 30 min for quinoline (Figure 66 and 67). Therefore, the following experiments were performed under equilibrium conditions after 24 h. Water-contaminant solutions with various concentrations of contaminant were mixed with montmorillonite or aquifer material of the same amount.

The sorption of compounds can be described by the linear regression according to the Freundlich model:

$c_s = K_F \times c_{aq}^{\,n}$,

or by the linearized form: $\log c_s = n \log c_{aq} + \log K_F$,

where c_s is the amount sorbed (mg/kg) and c_{aq} the equilibrium concentration in the liquid phase (mg/L). The distribution coefficient (K_F) is a measure which characterizes the potential of a chemical to be sorbed to the material investigated. A high distribution factor indicates a great potential of a compound for sorption to the studied sorbent. n characterizes the chemicals sorption behaviour. For $n > 1$ a potential increase of sorption isotherm appears, then sorption free energies are enhanced by increasing sorbate concentration. The contrary is the case for $n < 1$. Sorbates are bound weaker with increasing concentration. For $n = 1$ sorption free energies remain the same for all sorbate concentrations: then K_d- is identical to K_F-value (Schwarzenbach et al., 2003) and distribution coefficients (K_d [L/kg]) can be reached by $K_d = c_s / c_{aq}$.

Sorption isotherms of quinoline compounds could be described well by the linearized Freundlich model for montmorillonite as well as aquifer material as sorbent. Linear correlation coefficients (R^2) were higher than 0.92 for all compounds studied. K_F and n were taken from linear regression and are presented in Table 17 and 18.

A comparison of K_F-values from Freundlich plot and K_d-values of direct plots show a high accordance of data for the investigated hydroxylated compounds on montmorillonite as well as on aquifer material, while the deviation was highest for 6-quinolinol and quinoline on montmorillonite. For both compounds a high influence of concentration on K_d-value was indicated by the direct plot, which gets obvious by high standard deviation of K_d-values. At lowest concentration highest K_d-values were found (653 and 64 L/kg for 6-quinolinol and

quinoline, respectively), while K_d-values at highest concentration (100 mg/L) were found to be 43 and 20 L/kg, respectively. This dependency of concentration on sorption was absent for the other quinoline compounds investigated, but shows the limitations of the direct correlation. K_d-values were found to be independent on the range of concentration tested for sorption of quinoline compounds on aquifer material, as shown by the high accordance of K_d- and K_F-values.

Table 17: Parameters of the Freundlich isotherms on montmorillonite: n, K_F and R^2 and the corresponding pH-ranges. Furthermore, the K_d–value, derived as average of whole investigated concentration range is given.

compound	montmorillonite				
	n	K_F-value	R^2	K_d-value [L/kg]	pH-value
quinoline	0.74 ± 0.05	47.2 ± 6.5	0.96	32.3 ± 17.7	7.0-7.1
2(1H)-quinolinone	0.93 ± 0.02	3.0 ± 0.2	0.98	2.5 ± 0.8	7.4–7.5
3,4-dihydro-2(1H)-quinolinone	1.05 ± 0.04	1.9 ± 0.2	0.99	2.1 ± 0.5	7.4-7.5
1(2H)-isoquinolinone	0.97 ± 0.02	2.2 ± 0.1	0.99	2.1 ± 0.3	7.4-7.5
6-quinolinol	0.62 ± 0.02	170 ± 11	0.97	146 ± 120	7.0-7.1
4(1H)-quinolinone	0.96 ± 0.02	1.8 ± 0.1	0.99	1.7 ± 0.4	7.3-7.4

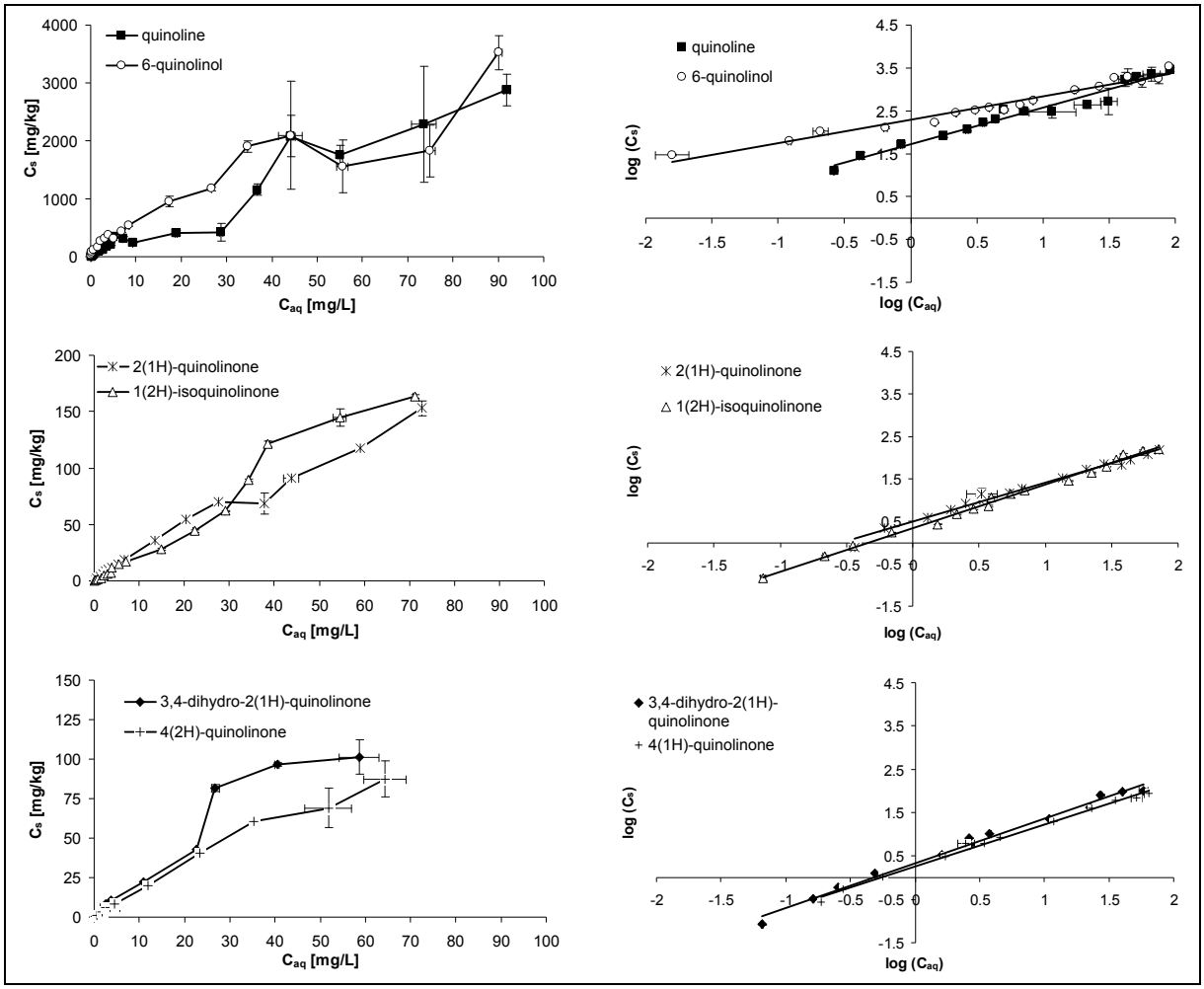


Figure 39: Sorption isotherms of quinoline compounds on K^+-montmorillonite. Plot of concentration sorbed/ concentration in aqueous solution (left hand side); isotherms according to Freundlich (right hand side).

Table 18: Parameters of the Freundlich isotherms on aquifer material and K_d-values.

compound	aquifer material				
	n	K_F-value	R^2	K_d-value [L/kg]	pH-value
quinoline	1.16 ± 0.05	0.25 ± 0.03	0.98	0.16 ± 0.05	6.7-6.9
2(1H)-quinolinone	0.82 ± 0.03	0.37 ± 0.03	0.97	0.23 ± 0.08	6.7-7.0
3,4-dihydro-2(1H)-quinolinone	1.32 ± 0.09	<0.1	0.92	0.12 ± 0.05	6.7-7.0
isoquinoline*	n.d.	n.d.	n.d.	0.10 ± 0.02	7.1-7.2
1(2H)-isoquinolinone	1.22 ± 0.05	0.12 ± 0.01	0.99	0.20 ± 0.09	6.7-6.8
3,4-dihydro-1(2H)-isoquinolinone*	n.d.	n.d.	n.d.	0.10 ± 0.04	7.1-7.2
4-methylquinoline*	n.d.	n.d	n.d.	0.24 ± 0.04	7.1-7.3
4-methyl-2(1H)-quinolinone*	n.d.	n.d.	n.d.	0.34 ± 0.02	7.1-7.4
6-quinolinol	0.93 ± 0.03	0.58 ± 0.04	0.99	0.73 ± 0.14	6.7-6.9
4(1H)-quinolinone	1.02 ± 0.05	0.24 ± 0.03	0.98	0.34 ± 0.10	6.7-7.1

n.d. = not determined. K_d–values derived as average of whole concentration range investigated, with the exception of those compounds which are marked (*). For those compounds, K_d is the result of only one concentration tested (1 mg/L) in triplicate.

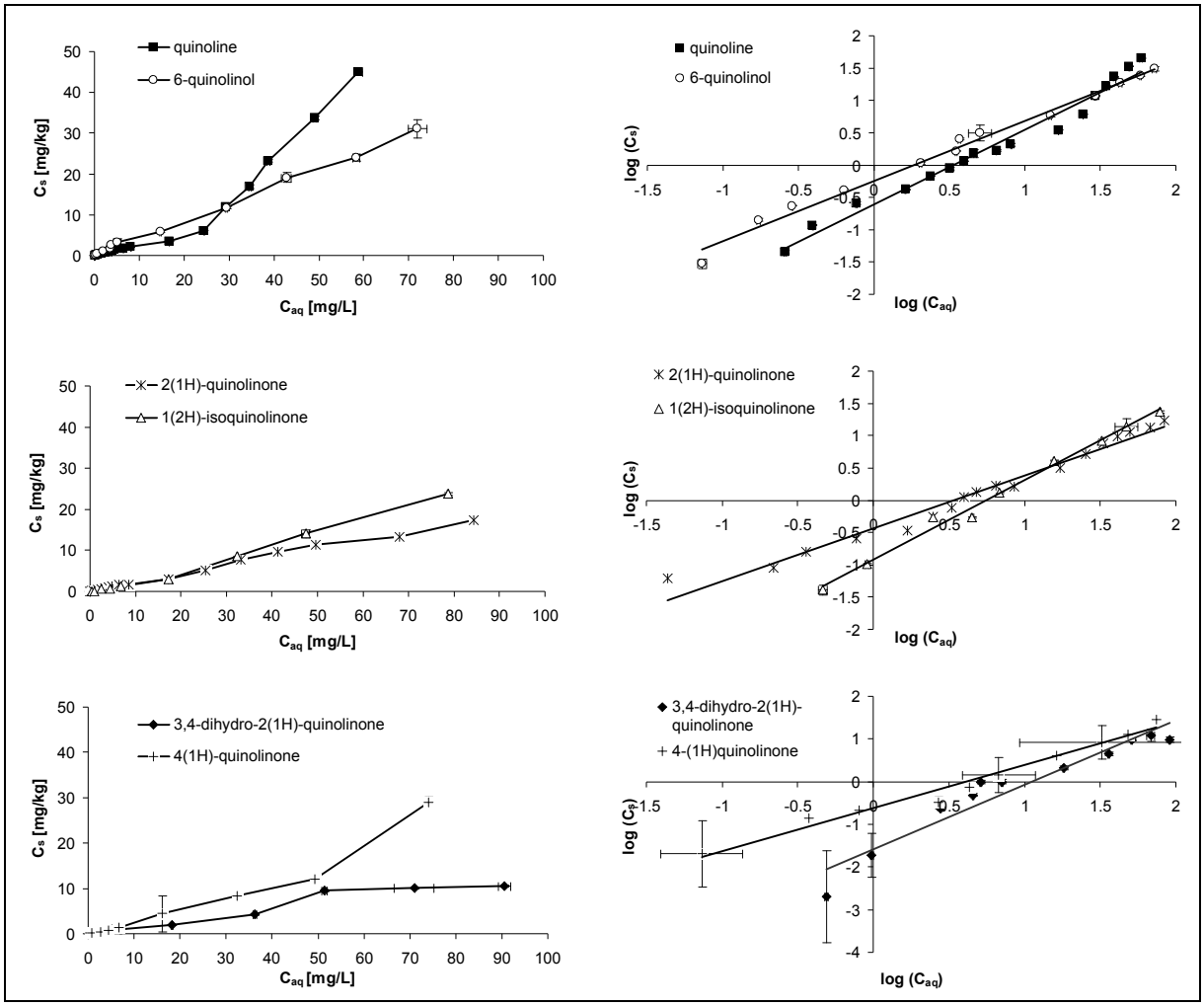


Figure 40: Sorption isotherms of quinoline compounds on aquifer material. Plot of concentration sorbed/ concentration in aqueous solution (left hand side); logarithmic plot according to Freundlich (right hand side).

Influence of pH on sorption

The investigated compounds can be divided into two groups according to their behaviour on pH-value: (1) quinoline and 6-quinolinol; (2) 2(1H)-quinolinone and 1(2H)-isoquinolinone.

(1) The graphs in Figure 41 show a strong correlation of pH- and K_d-values of quinoline and 6-quinolinol on montmorillonite as well as on aquifer material. K_d-values increased with rising acidity with a factor of more than 50 on montmorillonite. The K_d-value of quinoline increased from 20 at pH 9 to 872 at pH 3. K_d-value of 6-quinolinol increased from 14 to 1297 in the same pH-range. The K_d-value of 6-quinolinol did not increase in the range of pH 5.5-3.75, while sorption still increased with decreasing pH-value for quinoline.

Similar results were found for sorption on aquifer material. With decreasing pH the K_d of quinoline increased from 0.2 (pH 9.8) to 2.9 (pH 3.3) which is a progression of about a factor of 15. K_d–values of 0.04 to 6 were determined in the pH-range tested for 6-quinolinol.

(2) 2(1H)-Quinolinone and 1(2H)-isoquinolinone (Figure 41) showed low K_d–values of 3 L/kg, which remained constant over a wide range of pH. K_d-values of 2(1H)-quinolinone on aquifer material also showed minor influence on changes in pH-value; K_d increased with a factor of 2.4 from 0.14 to 0.36 L/kg.

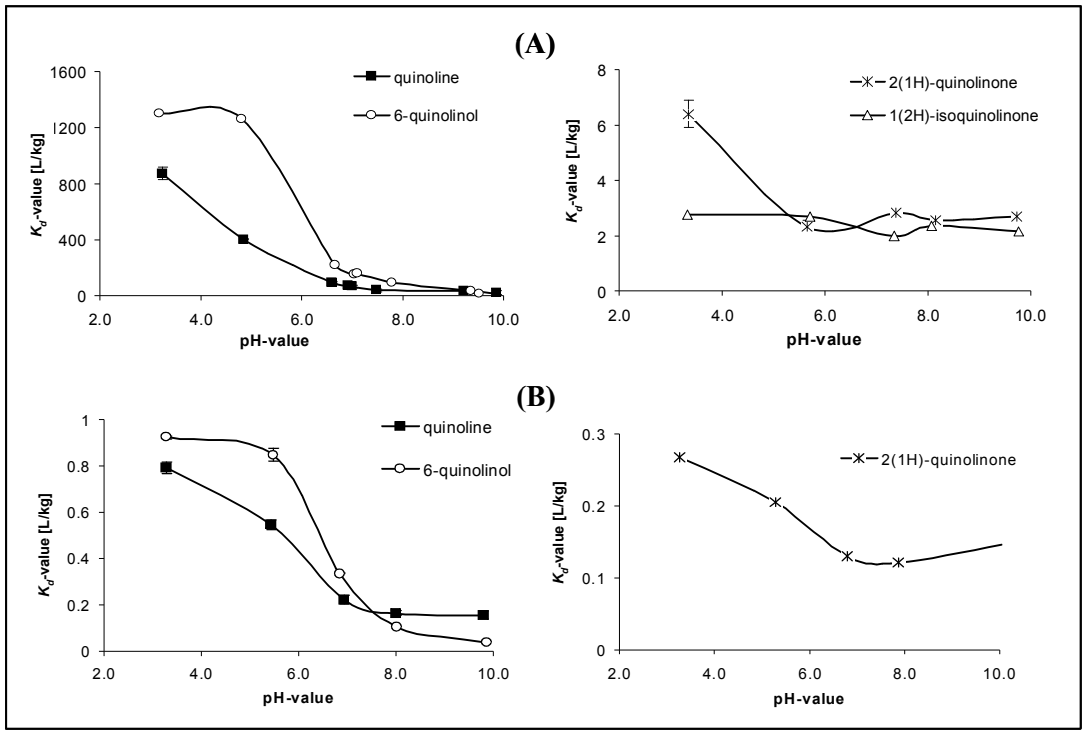


Figure 41: Influence of pH-value on sorption (K_d-value) of quinoline and 6-quinolinol as well as 2(1H)-quinolinone and 1(2H)-isoquinolinone; (A) sorption on montmorillonite; (B) sorption on aquifer material. Data points are a result of triplicates.

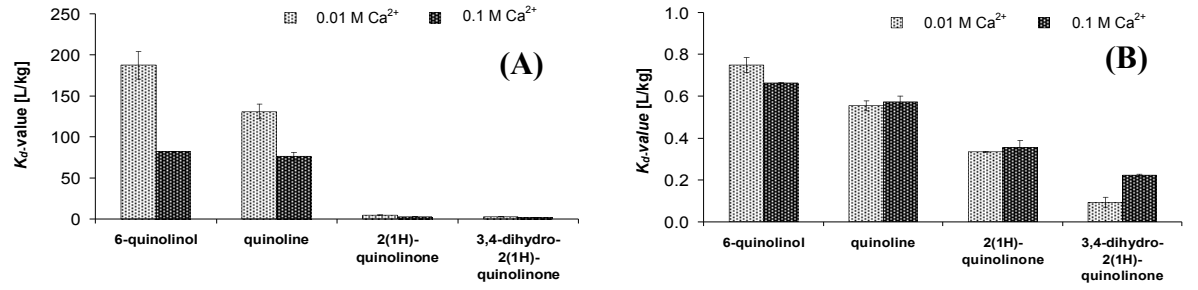
Influence of ionic strength on sorption

In this experiment the ionic strength was varied using synthetic groundwater with 0.01 M as well as 0.1 M $CaCl_2$. pH-values in all batches were 7.

The results showed a strong influence of ionic strength on sorption to montmorillonite. Results for quinoline, 6-quinolinol, 2(1H)-quinolinone as well as 3,4-dihydro-2(1H)-quinolinone are summarized in Figure 42. An increase of sorption of quinoline (factor 1.7) and 6-quinolinol (factor 2.3) is obvious with decreasing ionic strength. Even in case of

2(1H)-quinolinone as well as 3,4-dihydro-2(1H)-quinolinone an increase in sorption is remarkable (factor 1.6 and 1.3, respectively) which exhibit at lower ionic strength.

A variation of ionic strength in batches with aquifer material showed insignificant changes in sorption. Differences were within standard deviation (Figure 42 B).

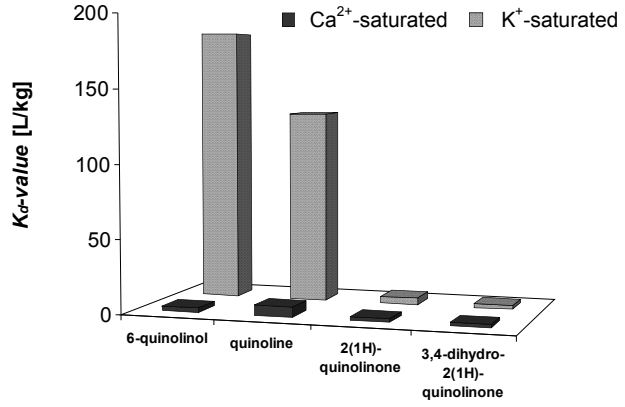


compound	ionic strength	ratio of sorbent/sorptive	montmorillonite K_d-value [L/kg]	aquifer material K_d-value [L/kg]
6-quinolinol	0.01 M Ca^{2+}	1:500	190 ± 17	0.75 ± 0.04
quinoline		1:500	131 ± 9	0.55 ± 0.02
2(1H)-quinolinone		1:5	4.6 ± 0.3	0.33 ± <0.01
3,4-dihydro-2(1H)-quinolinone		1:2	2.5 ± <0.01	0.09 ± 0.02
6-quinolinol	0.1 M Ca^{2+}	1:500	82.2 ± 0.5	0.66 ± <0.01
quinoline		1:500	76.4 ± 4.8	0.57 ± 0.03
2(1H)-quinolinone		1:5	2.8 ± 0.2	0.35 ± 0.04
3,4-dihydro-2(1H)-quinolinone		1:2	1.9 ± 0.1	0.22 ± <0.01

Figure 42: Influence of ionic strength of synthetic groundwater on sorption to montmorillonite (A), and aquifer material (B). The pH-value of the solution was at 7.2 ± 0.2 in batches with montmorillonite and at 6.7 ± 0.2 in batches with aquifer material. Error bars show standard deviations of three replicates.

Influence of exchangeable cations on sorption to montmorillonite

Montmorillonite was saturated with K^+ or Ca^{2+}-ions. Strong increase in sorption was observed for quinoline as well as 6-quinolinol, which showed 20-fold and 64-fold higher K_d-values on K^+-montmorillonite compared to Ca^{2+}-montmorillonite (both saturated with 1 M solutions) (Figure 43). Also for the less sorbing compounds 2(1H)-quinolinone (factor 2) and 3,4-dihydro-2(1H)-quinolinone (factor 1.3) a tendency to stronger sorption was found on K^+-montmorillonite than on Ca^{2+}-montmorillonite.



compound	cation	ratio of sorbent/sorptive	K_d-value [L/kg]
6-quinolinol	K^+	1:500	187 ± 17
quinoline		1:500	131 ± 9
2(1H)-quinolinone		1:5	4.6 ± 0.3
3,4-dihydro-2(1H)-quinolinone		1:2	2.7 ± <0.1
6-quinolinol	Ca^{2+}	1:500	2.9 ± 0.3
quinoline		1:500	6.7 ± 0.6
2(1H)-quinolinone		1:5	2.4 ± 0.1
3,4-dihydro-2(1H)-quinolinone		1:2	2.1 ± 0.1

Figure 43: Influence of the exchangeable cation (K^+ or Ca^{2+}) on sorption of quinoline compounds to montmorillonite; pH-value of solution was 7.2 ± 0.2. Three replicates were performed.

Discussion on sorption behaviour of quinoline compounds

The sorption of quinoline compounds to montmorillonite and aquifer material is a fast process (10-30 min). A fast equilibrium of sorption was also described in recent literature. Vasudevan et al. (2001) reported an equilibrium time of 1-2 min for sorption of N-heterocycles to metal oxides, while an equilibrium of sorption for quinoline to silica within 30 min was described by Zachara et al. (1990a).

An analysis of hydroxylated quinoline compounds shows a strong influence of the position of the OH-group on sorption properties. In Figure 44 it is illustrated that the position of a OH-group in the quinoline molecule has a strong effect on acidic-basic properties (pK_a-value).

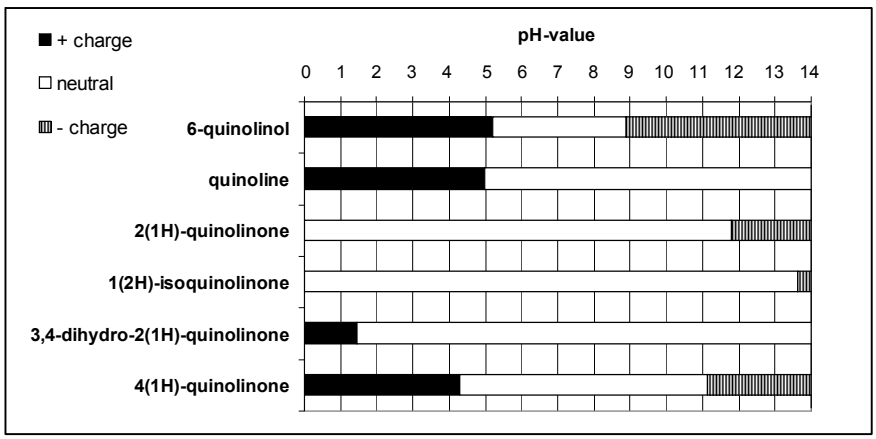


Figure 44: Formation of charges of quinoline compounds with respect to pH-value (according to Vasudevan et al., 2001).

The OH-substituent within the non-substituted ring in 6-quinolinol can only be present in the hydroxyl-form. All the other quinoline compounds are oxygenated in the *ortho*- or *para*-position to the N-atom and therefore may occur in either the oxo- or the hydroxylated form due to tautomeric interconversion (see Figure 4). Hence, the pK_a-values are shifted to extreme pH-values. However, in the pH-range studied here, these compounds (2(1H)-quinolinone, 1(2H)-isoquinolinone, 3,4-dihydro-2(1H)-quinolinone) are present in the neutral form, and therefore electrostatic interactions in sorption may be excluded. Only the pK_a-values of quinoline, 6-quinolinol and 4(1H)-quinolinone are within the investigated pH-range and these compounds are influenced by changes in pH. Considering these characteristics, the quinoline compounds resemble two groups according to their physicochemical behaviour and found differences in sorptive experiments showing alternative sorption mechanisms and intensity in sorption:

(1) charged organic compounds: quinoline, 6-quinolinol and 4(1H)-quinolinone

(2) uncharged organic compounds: 2(1H)-quinolinone, 1(2H)-isoquinolinone and 3,4-dihydro-2(1H)-quinolinone

The pK_a-values of quinoline, 6-quinolinol and 4(1H)-quinolinone are 5.0, 5.2 and 4.3, respectively (SciFinder, 2006). If the pH is below the pK_a, the N-protonated species is predominant. The pH-experiment shows that a correlation of pH-value and sorption intensity exists: increasing amounts of organic cation of the organic bases lead to higher sorption intensity. Cationic sorption is observed on montmorillonite as well as on aquifer material from the Castrop-Rauxel site. The same results were reported by Thomsen et al. (1999) and Zachara et al. (1986; 1988). Zwiener and Frimmel (2001) reported a strong influence of pH on sorption of 4(1H)-quinolinone to montmorillonite. The preferential sorption of 4(1H)-quinolinone was explained by the cationic form.

Quinoline sorption was found to be influenced by the cationic form in the present study as well as described in the literature, even up to 2 units higher than pK_a, which may be explained by increasing concentrations of protons near the surface of minerals, resulting in local pH-values up to 2 units lower. Furthermore, the stronger sorption of the cationic species even in minor concentrations might lead to noticeable sorption in contrast to the uncharged species.

The aquifer material, which mainly consists of sand fractions, also allows ionic sorption as has been found in the pH-experiment for quinoline and 6-quinolinol. In addition to the clay fraction present (0.25 %), silica also represents a potential subsurface sorbent of organic bases because its surface contains weakly acidic silanol groups that are negatively charged above pH 5 (Abendroth et al., 1970; Schindler et al., 1968). Zachara et al. (1990a) reported an increase in sorption of quinoline with decrease in pH, while there was no effect found due to the protonation of silanol groups. The minor influence of pH on sorption of 2(1H)-quinolinone and 1(2H)-isoquinolinone to montmorillonite and aquifer material can be explained by the very low pK_a-values (-0.41 and -1.20; SciFinder, 2006). Therefore, these compounds are uncharged in groundwater with an average pH of 6.9 as well as in the tested pH-range of 3.3–9.9. Some slightly higher sorption of 2(1H)-quinolinone to montmorillonite and aquifer material was determined at the lowest pH-value investigated (pH 3.3), which may be due to a starting protonation.

The results obtained by varying the ionic strength of the surrounding water phase at pH 7 support the assumption that sorption of the two investigated ionizable compounds, quinoline and 6-quinolinol to montmorillonite is explainable due to the cationic form. A higher sorption was found at low ionic strength. Higher sorption of the organic cations of quinoline or 6-quinolinol is influenced by the competition of organic and inorganic cations for sorption sites on the negative charged surface of montmorillonite. When using aquifer material as sorbent, the influence of ionic strength on sorption was found to be low or absent. However,

generally the intensity in sorption was low. Sorption of uncharged species at neutral pH via hydrogen bonding or van der Waals forces might be considerable processes.

The variation in montmorillonite saturated with a monovalent (K^+) or divalent cation (Ca^{2+}) showed higher sorption on K^+-saturated montmorillonite towards ionizable as well non-ionizable quinoline compounds. However, the intensity in sorption was found to increase much stronger for ionizable compounds indicating the influence of cationic sorption besides the presence of other factors, which are independent on electrostatic interactions.

Sorption of ionizable as well as non-ionizable organic compounds was frequently demonstrated to be strongest on K^+-saturated clay minerals (Li et al., 2004). The sorption of a cation to the mineral surface is a result of electrostatic interactions. The hydratation of K^+ (- 314 kJ/mol) is relatively weak compared to the hydratation of a multivalent cation like Ca^{2+} (-1580 kJ/mol) (Li et al., 2004). If the surface of a clay mineral is saturated with a strongly hydrated divalent cation like Ca^{2+}, the hydratation sphere surrounding the exchangeable cations diminishes the size of adsorptive domains between the cations and reduces the strength of interactions between exchangeable ions and the organic contaminant. Additionally, the nanostructure of clay quasicrystals formed may play an important role in determining the affinity of organic compounds (Li et al., 2004). Because of its lower charge, the entrance of a monovalent cation into the interplatelet region of the mineral is accompanied by the addition of several layers of water and results in the propping open of the interplatelet regions. Ca^{2+} as bivalent cation promotes the formation of quasicrystals with more than 20 platelets, while K^+ forms up to 3 platelets/quasicrystal (Verburg and Braveye, 1994). Accordingly, the accessibility of sorption sites is higher in K^+-montmorillonite compared to Ca^{2+}-montmorillonite. The basal spacing of ~12.3 Å is thought to be optimal for the sorption of organic contaminants (Sheng et al., 2002; Laird et al., 1992). The spacing is ideal for intercalation of the contaminant, while the interaction with water molecules is minimized. This process is found to occur, when the aromatic compound directly contacts the opposing clay siloxane surface. The dehydration of the organic contaminant in the clay interlayers is not allowed for larger interlayer spacings associated by multivalent exchangeable cations like Ca^{2+} (Sheng et al., 2002; Boyd et al., 2001; Li et al., 2003; Johnston et al., 2002). Therefore, the better exchangeability of a monovalent cation, the formation of more accessible surface areas due to the swelling of the mineral as well as the formation of ideal spacings in K^+-saturated smectites are factors explaining the higher sorption for both groups of compounds ionizable as well as non-ionizable quinoline compounds on K^+-montmorillonite.

Furthermore, the results obtained with 6-quinolinol showed that its sorption is highly affected by the presence of the hydroxyl-function. In addition to the electrostatic interaction of the protonated N-atom, the OH-group may also interact with the surface (metal oxides) via electrostatic forces and surface complexation. Furthermore, electron-donor-acceptor interactions (hydrogen bonding) may influence the sorption intensity. Hydrogen bonding of the N^+-H-group or an OH-group linked to the oxygen on the silica or clay surface or to surface-bound water leads to a further process of sorption. These effects are relevant as shown by the higher affinity of 6-quinolinol to montmorillonite and aquifer material compared to quinoline.

Conclusion – Transfer to the field site

Most studies on sorption of organic contaminants focussed on sorption on organic matter as primary sorptive domain. Sorption processes are in general dominated by this process. However, in aquifers characterized by low organic matter content, a situation found for the three sites studied here, sorption to mineral surfaces becomes the dominating sorption mechanism (Schwarzenbach and Westall, 1981; Banerjee et al., 1985; Piwoni and Banerjee,

1989; Hundal et al., 2001). In general, the results obtained with the material from the Castrop-Rauxel field site show low sorption for all quinoline compounds investigated. The aquifer material is characterized by low organic carbon content (<0.1 %) and low content of clay minerals (0.25 %) which are the main sorbents for organic compounds. Accordingly, K_d-values (K_F-values) of quinoline compounds were observed, which have to be classified as low for all compounds according the definition of Kukowski (1989). The pH-value of the groundwater at all of the field sites is within a range of 6.6-7.6. Therefore, a high difference in sorption due to the higher sorption intensity of the cationic fraction of quinoline and 6-quinolinol may not be of significance.The variance in sorption intensity of quinoline and its hydroxylated analogs 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone as well as isoquinoline and 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone is found to be negligible. The same is true for 4-methylquinoline and its hydroxylated analog 4-methyl-2(1H)-quinolinone.

Therefore, sorption will not influence the rate of transport of the parent compounds and their metabolites in the aquifer significantly.

4.3.3 Hydroxylated quinoline compounds as indication for natural attenuation

4.3.3.1 Presence of hydroxylated quinoline compounds in tar oil products

Microbial degradation experiments under anaerobic conditions (chapter 4.3.2.1) with aquifer material from the Castrop-Rauxel site showed the formation of hydroxylated metabolites. Therefore, the origin of hydroxylated compounds in microcosm experiments is clear. Hydroxylated quinoline compounds observed at contaminated sites are generally discussed as biotransformation products. However, Goerlitz et al. (1985) and Johansen et al. (1997b) speculated about the existence of quinolinones in the original tar oils, but conclusive data have not been reported until now. Other hydroxylated aromatic compounds such as 1- or 2-naphthol, 6(5H)-phenanthridinone, phenols, indanol and indenol have been reported as components of tar oils (Pawellek et al., 1979; Lang and Eigen, 1967).

Therefore, the analysis of tar oil products was performed using HPLC-MS-MS. The tar oils and coal tar investigated are todays products used for wood impregnation, furthermore commercially available products as wells as one tar oil originating from the source of a contaminated site. Since the original tar oils and tar products, which contaminated the sites a long time ago, were not available and would have been changed in composition due to environmental processes anyway, these actual tar products were used as substitutes. It is known that the processes of tar oil distillation have changed during the last decades, because of legal reasons directed to reduce the benzo-[a]-pyrene content (Commission directive 2001/90/EC). Therefore, the composition of these tar products could consequently only be used as an indication of the original composition.

The analyses of the seven different tar oils showed the presence of 2(1H)-quinolinone and 1(2H)-isoquinolinone as well as their reduced species 3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone. Furthermore, 4-methyl-2(1H)-quinolinone was found in every mixture (Table 19).

Table 19: Occurrence of quinoline and isoquinoline as well as the hydroxylated derivatives in various tar products.

tar product	2(1H)-quino-linone	3,4-dihydro-2(1H)-quino-linone	qui-noline	1(2H)-iso-quino-linone	3,4-dihydro-1(2H)-iso-quino-linone	iso-qui-noline	4-methyl-2(1H)-quino-linone	4-methyl-qui-noline	Σ methyl-2(1H)-quino-linone	Σ methyl-qui-noline
no.*	conc [mM]	conc [mM]	conc [mM]	conc [mM]	conc [mM]	conc [mM]	conc [mM]	conc [mM]	conc [mM]	conc [mM]
1	1.04	0.30	0.38	0.73	0.01	0.22	0.30	0.21	1.11	0.66
2	1.84	0.32	2.47	1.13	0.02	1.11	0.54	1.14	2.24	2.83
3	1.66	0.46	4.13	1.18	0.03	2.53	0.60	2.42	2.92	5.44
4	0.81	0.33	0.34	0.72	0.04	0.16	0.24	0.20	1.26	1.81
5	0.30	0.02	21.3	0.21	<0.01	7.62	0.10	2.70	0.50	17.1
6	6.99	0.07	24.6	0.35	<0.01	9.42	1.26	6.99	3.2	16.6
7	0.15	0.12	2.06	0.07	<0.01	0.88	0.04	0.41	0.15	1.00

* see tar product description in chapter 3.8.

These data clearly indicate that the detection of the hydroxylated and hydrogenated quinolines in the groundwater is not a sufficient indicator for the occurrence of biological natural attenuation processes in the field sites.

4.3.3.2 Ratio approach of hydroxylated/parent compound

The results on the presence of hydroxylated quinoline compounds in the tar products showed that the hydroxylated quinoline derivatives are useless as stand-alone indicators for biodegradation in the field.

As an alternative idea on the presence of natural attenuation, the molar ratio of 2(1H)-quinolinone and/or 3,4-dihydro-2(1H)-quinolinone to quinoline as well as the isomeric compound couple 1(2H)-isoquinolinone and/or 3,4-dihydro-1(2H)-isoquinolinone to isoquinoline, named $R_{metabolite}$ is proposed here as an indicator for microbial NA processes:

$$R_{metabolite} = \frac{c\ (\text{hydroxylated compound or reduced and hydroxylated compound})}{c\ (\text{parent compound})}$$

It was tested here, if a comparison of the ratios in tar oils and in the field sites may give hints for natural attenuation. However, all important factors which can influence $R_{metabolite}$ have to be recognized and compared to conclude about natural attenuation in the field.

These are:

(1) dilution,

(2) volatilization,

(3) microbial degradation,

(4) sorption, and

(5) dissolution from tar products to groundwater.

(1) If one assumes that dilution is the only process at the site, the same $R_{metabolite}$ would be present all over the plume, because all compounds are influenced the same way.

(2) Volatilization of quinoline, isoquinoline and hydroxylated quinoline and isoquinoline compounds can be expected to be insignificant in groundwater, because Henry's law constants are very low (in the range of 10^{-10}-10^{-7} atm × m^3/mol) (Table 22) (U.S. EPA. 2000; Mackay and Yuen., 1980).

(3) The degradation experiments with aquifer material from the Castrop-Rauxel site showed that the 2(1H)-quinolinone, 1(2H)-isoquinolinone and 3,4-dihydro-2(1H)-quinolinone are products of microbial degradation of quinoline and isoquinoline. Thus, microbial degradation influences $R_{metabolite}$. Different scenarios for the outcome are possible:

(a) The formation of a stable metabolite from quinoline or isoquinoline will result in an increasing concentration of 2(1H)-quinolinone or 1(2H)-isoquinolinone and a decreasing concentration of the parent compound. Consequently, $R_{metabolite}$ will increase.

(b) If the metabolite is degradable, any differences in degradation rates for metabolite and parent compound would cause a change in $R_{metabolite}$ in the plume. The changes of $R_{metabolite}$ can either be an increase (quinoline transformation is faster than 2(1H)-quinolinone degradation) or a decrease (rate of quinoline transformation is lower than transformation of 2(1H)-quinolinone).

(4) Differences in sorption behaviour of 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone and quinoline as well as 1(2H)-isoquinolinone, 3,4-dihydro-2(1H)-quinolinone and isoquinoline may shift the ratios in the contamination plume.

Since the pH-values of the aquifer at the three field sites Castrop-Rauxel, Düsseldorf-Flingern and Wülknitz are in the range of 6.6-7.6, specific sorption of the cationic form of quinoline and isoquinoline will play a minor role and no remarkable differences between sorption intensity of quinoline and hydroxylated quinoline and isoquinoline compounds will be expected as studied in chapter 4.3.2.2. In addition, due to the high sand content of the aquifer material with carbon contents lower than <0.1-0.9 % and low mineral fraction of 0.25-5.5 % at all three investigated sites, sorption is assumed to be of minor significance.

K_d-values of 0.16 ± 0.05, 0.23 ± 0.08 and 0.12 ± 0.05 L/kg for quinoline, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone and K_d-values of 0.10 ± 0.02, 0.20 ± 0.09 and 0.10 ± 0.04 L/kg for isoquinoline, 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone with aquifer material from the Castrop-Rauxel site were determined (chapter 4.3.2.2). The differences of sorption of parent compounds and hydroxylated analogs is statistically insignificant.

Furthermore, aquifer material from the Castrop-Rauxel site was extracted, which derived from one of the highest contaminated wells. Comparing the relative concentrations of quinoline as well as 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone in aquifer material and groundwater of well T12, similar $R_{metabolite}$ were observed with both matrices: $R_{2(1H)\text{-quinolinone}}$ and $R_{3,4\text{-dihydro-}2(1H)\text{-quinolinone}}$ were 45 and 0.3 (aquifer material) and 49 and 2 (groundwater), respectively. The same was found for the isomeric $R_{metabolite}$: $R_{1(2H)\text{-isoquinolinone}}$ and $R_{3,4\text{-dihydro-}1(2H)\text{-isoquinolinone}}$ were 7 and <0.1 (aquifer material) as well as 22 and 0.4 (groundwater). This confirms the assumption that sorption and desorption of these compounds occur in a similar range and sorption is not a dominant mechanism shifting $R_{metabolite}$ to higher values in the aquifer.

(5) Differential dissolution of parent compounds and metabolites from the tar product has to be taken into account as well. When a multiple component mixture like tar oil is in contact with water, the most soluble components are depleted fastest from the NAPL source (nonaqueous phase liquids). The dissolved equilibrium concentrations may be estimated by the Raoult´s law: $C_i = x_i \times S_i$,

where C_i is the aqueous equilibrium concentration of compound i, x_i is the mole fraction of compound i in the NAPL and S_i is the saturation concentration of compound i in water.

However, the Raoult´s law is accessible for apolar compounds in mixture in which the major components undergo primarily van der Waals interactions as well as for monopolar compounds with the restriction that in the mixture there are no major constituents exhibiting a significant complementary polarity. For PAHs from coal tars or tar oils, the applicability of Raoult´s law was indicated within a factor 2-3 (Schwarzenbach et al., 2003). For bipolar compounds, which are capable of H-donor or H-acceptor interactions, such as phenolic compounds or aromatic amines, larger deviations from the ideal behaviour were found (Schmidt et al., 2002).

Assuming ideal behaviour of compounds and a molar mass of 150 g/mol of tar products (Schwarzenbach et al, 2003) the application of Raoult´s law shows an $R_{2(1H)\text{-quinolinone}}$ of 0.5 in the water phase, when the tar oil with the highest $R_{metabolite}$ of 3 was used for the calculation. So the ratio in the water phase would be approximately 6 times lower than the ratio in the tar oil.

Additional batch experiments were carried out here to verify the influence of dissolution from the oil to the water phase on $R_{metabolite}$. Results shown as $K_{tar\ oil/water}$ are summarized in Table

20. In contrast to values calculated by the Raoult´s law, an approx. 10 times higher dissolution of 2(1H)-quinolinone and 1(2H)-isoquinolinone was found compared to quinoline and isoquinoline, respectively. This correlates with the lower log P_{OW} values of the quinolinones compared to the parent compounds. Correspondingly, this dissolution behaviour shifts $R_{metabolite}$ in the groundwater to 10 times higher ratios.

Table 20: Partitioning coefficients between tar oil and water phase for quinoline derivatives.

compound	$K_{tar\ oil/\ water}$*
quinoline	3.52 ± 0.42
2(1H)-quinolinone	0.32 ± 0.05
3,4-dihydro-2(1H)-quinolinone	0.45 ± 0.26
isoquinoline	3.46 ± 0.63
1(2H)-isoquinolinone	0.28 ± 0.03
3,4-dihydro-1(2H)-isoquinolinone	0.29 ± 0.03
4-methylquinoline	17.0 ± 3.55
4-methyl-2(1H)-quinolinone	1.25 ± 0.14
Σ methylquinoline	15.8 ± 2.13
Σ methyl-2(1H)-quinolinone	3.85 ± 0.43

* determined by HPLC-DAD, see chapter 3.3.1 as well as 3.10; 3 three tar oils were investigated, each in triplicate.

Application of the ratio hydroxylated/parent compound

When looking at ratios of concentration in tar oil products between 2(1H)-quinolinone or 3,4-dihydro-2(1H)-quinolinone and quinoline as well as those between 1(2H)-isoquinolinone or 3,4-dihydro-2(1H)-quinolinone and isoquinoline, respectively, certain variability is found (Table 21). The molar ratios of 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone in relation to quinoline vary from 0.01 to 2.75 and <0.01 to 0.99, respectively. For the molar ratios of 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone related to isoquinoline a variation within 0.04 to 4.46 and <0.01 to 0.22, respectively, was found.

Table 21: Ratio ($R_{metabolite}$) of quinoline compounds in tar products.

tar product	2(1H)-quinolinone / quinoline	3,4-dihydro-2(1H)-quinolinone / quinoline	1(2H)-iso-quinolinone / isoquinoline	3,4-dihydro-1(2H)-iso-quinolinone / isoquinoline	4-methyl-2(1H)-quinolinone / 4-methyl-quinoline	Σ methyl-2(1H)-quinolinone / Σ methyl-quinoline
no.	$R_{metabolite}$	$R_{metabolite}$	$R_{metabolite}$	$R_{metabolite}$	$R_{metabolite}$	$R_{metabolite}$
1	**2.75**	0.78	3.29	0.06	**1.46**	**1.70**
2	0.75	0.13	1.02	0.02	0.47	0.79
3	0.40	0.11	0.47	0.01	0.25	0.54
4	2.40	**0.99**	**4.46**	**0.22**	1.17	0.70
5	0.01	<0.01	0.03	<0.01	0.04	0.03
6	0.28	<0.01	0.04	<0.01	0.18	0.19
7	0.07	0.06	0.08	<0.01	0.09	0.15
median	0.40	0.11	0.47	0.02	0.25	0.54

The ratios are related to parent compounds. Largest ratios are marked bold. Furthermore, median values of the seven tar products are given.

A comparison of ratios is schematically shown in Figure 45, where $R_{metabolite}$ of field data from Castrop-Rauxel, Düsseldorf-Flingern as well as Wülknitz are compared to those $R_{metabolite}$ of tar products in form of a box plot. Only those ratios were included, where both compounds, metabolite and parent compound, were present in concentrations higher than the limit of detection.

Castrop-Rauxel site. A clear direction of the shift of $R_{metabolite}$ is not observed downstream the plume. There exists some variation of $R_{metabolite}$ in groundwater of the different wells and also in different depth. An inhomogenous distribution of contaminants in the plume might result from the presence of non-dissolved tar products in the aquifer material (Schnier, 1999). The observed $R_{metabolite}$ in groundwater samples of the testfield exceeds the highest ratio found in tar products in 65 % for $R_{2(1H)\text{-quinolinone}}$, in 76 % for $R_{1(2H)\text{-isoquinolinone}}$, in 80 % for $R_{3,4\text{-dihydro-2(1H)-quinolinone}}$ and in 83 % for $R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$. Taking into account the different dissolution of parent compounds and metabolites from the tar oil into the water phase with a factor of 10, the percentage of groundwater samples exceeding maximum $R_{metabolite}$ is still 23 % for $R_{2(1H)\text{-quinolinone}}$, 31 % for $R_{1(2H)\text{-isoquinolinone}}$, and 19 % for $R_{3,4\text{-dihydro-2(1H)-quinolinone}}$. For $R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$ none of the analysed samples exceed the maximum ratio. Therefore, in several wells the ratios clearly exceed the $R_{metabolite}$ for most compound couples even if dissolution from tar products to the water phase is included. For instance, including the tenfold higher dissolution of metabolites to groundwater than parent compounds, the highest $R_{2(1H)\text{-quinolinone}}$ of 115 which was found in well T8 (5 m) in the middle of the testfield, is 4-fold of highest ratio in tar oil products. In case of 3,4-dihydro-2(1H)-quinolinone the largest $R_{metabolite}$ of 34 was found in well T9 (7 m) (4-fold highest ratio). For 1(2H)-isoquinolinone the largest $R_{metabolite}$ reached 599 (11-fold highest ratio) in well T15 (5 m).

The different ratios vary over the field site. There is no clear area present, where all ratios show a maximum. The $R_{2(1H)\text{-quinolinone}}$ varied within a range of 0.2–115. The range for $R_{1(2H)\text{-isoquinolinone}}$, $R_{3,4\text{-dihydro-2(1H)-quinolinone}}$, and $R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$ was 1-599, 0.1–34, and 0.1-2, respectively.

Düsseldorf-Flingern site. When looking at the ratios of hydroxylated quinoline/isoquinoline and parent compounds obviously the highest values were present in lowest depths. With increasing depth of the groundwater analysed the ratios decreased. Significantly, quinoline was absent in various wells downstream, while isoquinoline was still present.

Highest ratios were found in line 1 in wells 19200 and 19201 for $R_{2(1H)\text{-quinolinone}}$ 36.4, $R_{3,4\text{-dihydro-2(1H)-isoquinolinone}}$ 13.5, and $R_{1(2H)\text{-isoquinolinone}}$ 696, while $R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$ was only 0.2. The ratio of 1(2H)-isoquinolinone/isoquinoline represented the highest one, a result known from the Castrop-Rauxel site. In several wells the ratios clearly exceeded the $R_{metabolite}$, when dissolution from tar products to the water phase was included, i. e. the tar oil ratios were multiplied by a factor of 10. The percentage of groundwater samples exceeding this threshold ratio were 7 % for $R_{2(1H)\text{-quinolinone}}$, 9 % for $R_{1(2H)\text{-isoquinolinone}}$, 5 % for $R_{3,4\text{-dihydro-2(1H)-quinolinone}}$. In no groundwater sample the $R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$ reached the highest ratio found in tar oils.

Wülknitz site. At the Wülknitz site all the ratios calculated exceeded the highest value found in the tar oils. Generally, as has been observed at the other sites, some variation of $R_{metabolite}$ in groundwater of the different wells was determined. Since only a restricted number of values was available from the Wülknitz site, a statistical evaluation was not possible.

In most of the wells far away from the source (well 14/01 and 6/98) the parent compounds were present below the limit of detection. When both compounds were present highest values of ratios were found in the wells with highest contamination.

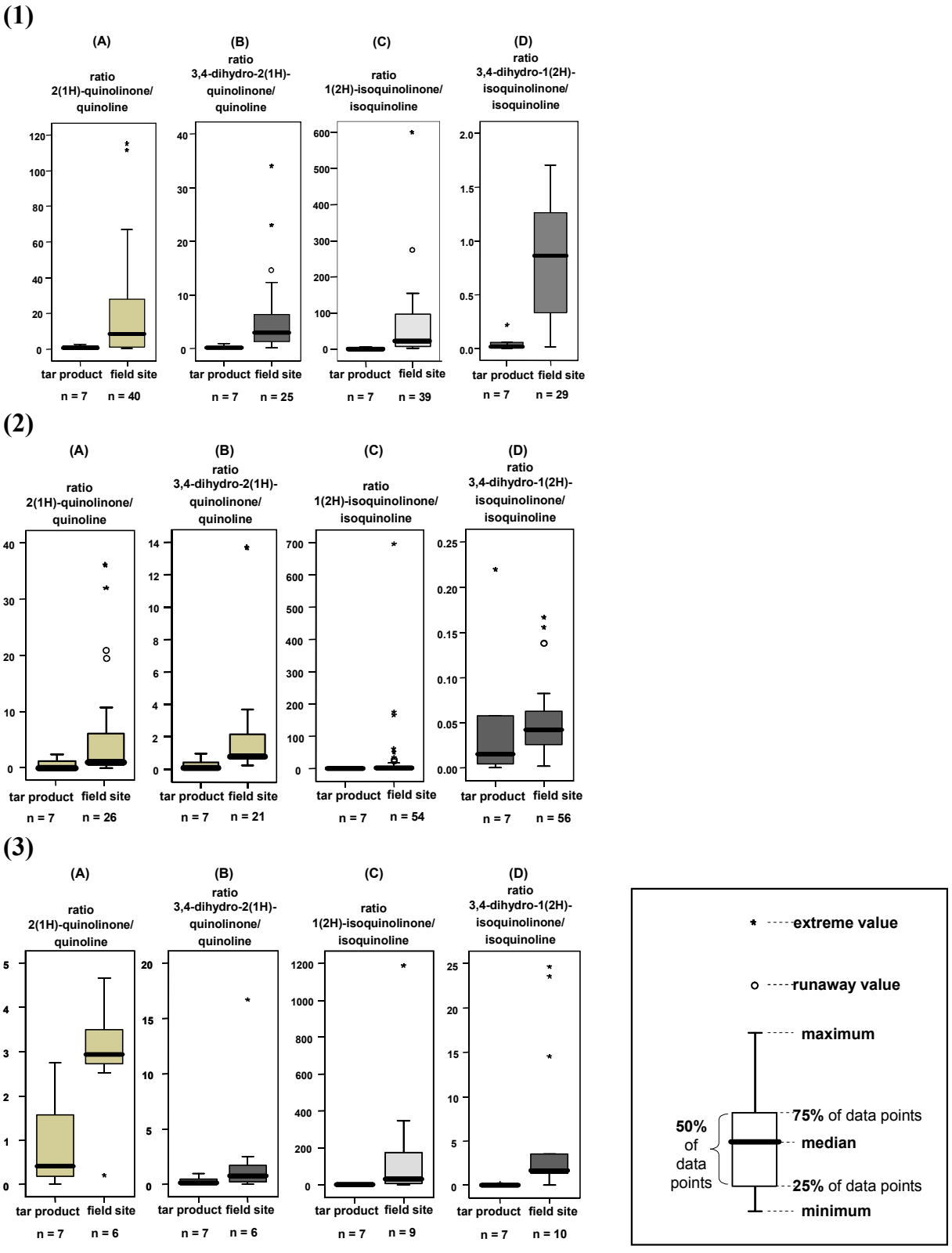


Figure 45: Box plots of $R_{metabolite}$. (A) 2(1H)-quinolinone/quinoline, (B) 3,4-dihydro-2(1H)-quinolinone/quinoline, as well as (C) 1(2H)-isoquinolinone/isoquinoline and (D) 3,4-dihydro-1(2H)-isoquinolinone/isoquinoline in tar products and groundwater samples from (1) the Castrop-Rauxel, (2) the Düsseldorf-Flingern, and (3) the Wülknitz site. Note high differences in scales!

The data are presented as follows: extreme value: more than 3 length of boxes outside the box; runaway value: distance to box more than 1.5-3 length of box; n = number of data points; ratios were only calculated if both compounds were present in concentrations higher than limit of detection.

In contrast to the Castrop-Rauxel and Düsseldorf-Flingern site $R_{2(1H)\text{-quinolinone}}$ reached only a value of 5 in maximum, while the maximum of $R_{3,4\text{-dihydro-}2(1H)\text{-quinolinone}}$ with 17 was in a similar range. Ratios of 25 and 1188 for $R_{3,4\text{-dihydro-}1(2H)\text{-isoquinolinone}}$ and $R_{1(2H)\text{-isoquinolinone}}$, respectively, were found to be even higher than at both other sites.

The threshold value, obtained from the tar oil ratio multiplied by the dissolution factor of 10, was never exceeded for the $R_{2(1H)\text{-quinolinone}}$. However, the other compound couples clearly rise above this threshold value in many samples: 56 % for $R_{1(2H)\text{-isoquinolinone}}$, 17 % for $R_{3,4\text{-dihydro-}2(1H)\text{-quinolinone}}$, and 40 % for $R_{3,4\text{-dihydro-}1(2H)\text{-isoquinolinone}}$.

Feasibility of the ratio approach using methylsubstituted quinolines

Methylquinolines were found to be more widespread distributed over the three field sites than quinoline itself. While quinoline concentrations decreased fast with the groundwater flow, methylquinolines were often still present downstream. The feasibility of the ratio approach was investigated for the couple 4-methyl-2(1H)-quinolinone and 4-methylquinoline. This implied the advantage that a higher number of values was available for the determination of ratios. In addition, the ratio of the sum of methyl-2(1H)-quinolinones and methylquinolines was calculated. 4-Methyl-2(1H)-quinolinone is the only methyl-2(1H)-quinolinone, which is commercially available until now. It was used as standard compound for the quantification of all the other isomers found which show a similar transition in MS-MS. Although chromatographic separation of isomers was limited and a clear identification of isomers was complicated the ratio of the sum of compounds was formed.

As has been found for non-substituted quinoline and isoquinoline compounds the ratios of methylquinolines in tar oils varied in a high extent, within 0.04 to 1.46 for the 4-methylisomer couple and <0.03 to 1.70 for the sum of all methyl-isomers (Table 21). The differences in dissolution from tar oil phase into the water phase (Table 20) let to 14-fold higher concentrations of hydroxylated analogs in the water phase than methylquinolines. By paying attention to the maximum ratio which can occur without any microbial activity, a high number of samples at all of the sites clearly exceeded the threshold (Figure 46). At the Castrop-Rauxel site 7 and 75 % of the samples exceeded $R_{4\text{-methyl-}2(1H)\text{-quinolinone}}$ and $\Sigma R_{\text{methyl-}2(1H)\text{-quinolinone}}$, respectively. For samples of Düsseldorf-Flingern 24 % of samples exceeded the threshold for $R_{4\text{-methyl-}2(1H)\text{-quinolinone}}$, and 5 % for $\Sigma R_{\text{methyl-}2(1H)\text{-quinolinone}}$, may be probably indicating a better degradability of 4-methylquinoline or less degradability of 4-methyl-2(1H)-quinolinone. At the Wülknitz site both ratios were exceeded in a high number (27 % for $R_{4\text{-methyl-}2(1H)\text{-quinolinone}}$ and 60 % for $\Sigma R_{\text{methyl-}2(1H)\text{-quinolinone}}$) of samples.

These results show that the ratio approach is also feasible for methylquinolines. Also here, clear hints for natural attenuation could be worked out.

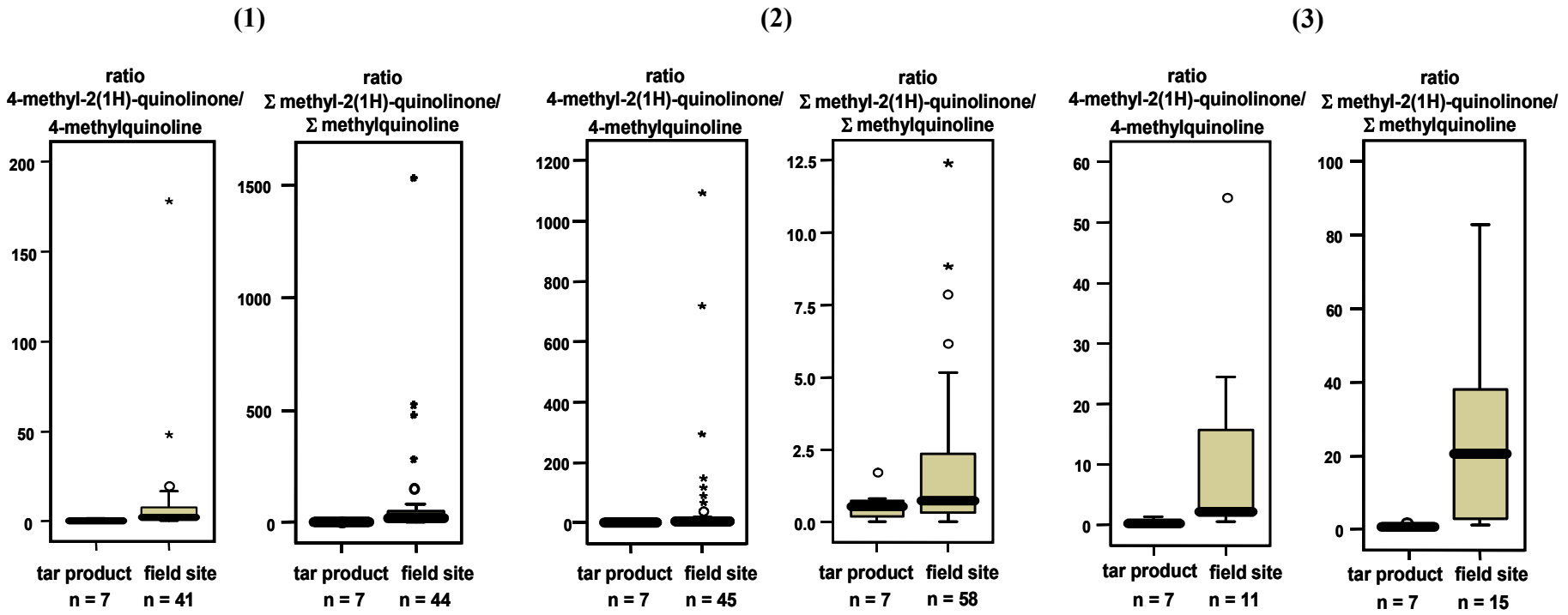


Figure 46: Box plots of $R_{metabolite}$: 4-methyl-2(1H)-quinolinone/4-methylquinoline as well as Σ methyl-2(1H)-quinolinone/Σ methylquinoline in tar products and groundwater samples from the (1) the Castrop-Rauxel, (2) the Düsseldorf-Flingern and (3) the Wülknitz site.

4.3.3.3 Quinolines as indicator for natural attenuation: Discussion and conclusion

The data presented in this chapter demonstrate that it is important to collect information about the composition of tar products to avoid misleading conclusions about metabolites. In addition, it was found that it is insufficient to solely analyse the fate of metabolites at a contaminated site when searching for natural attenuation. In fact, a side-by-side analysis of the occurrence of parent compounds and metabolites and their comparison expressed here as $R_{metabolite}$ indicates *in situ* biodegradation at the field site, if the ratio exceeds a threshold factor which is higher than the ratio within typical tar oil products including differences in distribution.

The two parameters influencing the ratio of metabolite and parent compound in the aquifer are dissolution from tar oils as NAPLs into the water phase as well as biodegradation. Differences in sorption will influence the ratio only to a minor extend. Multiplying the highest ratio found in tar oils with a factor of 10, differences in dissolution of hydroxylated compounds with parent compounds are considered. Therefore, higher ratios found at the field site are only explainable by biodegradation of quinoline compounds, a result found at the three sites for the most ratio couples established. The microcosm experiments (chapter 4.3.2.1) clearly indicate that the hydroxylation of quinoline or isoquinoline led to the accumulation of hydroxylated analogs. The studies of the degradation of quinoline, 2(1H)-quinolinone, and 3,4-dihydro-2(1H)-quinolinone in microcosms showed that the hydroxylation of quinoline was a rapid microbial process and the further transformation of 2(1H)-quinolinone was a slow one, so that high amounts of 2(1H)-quinolinone accumulated. 3,4-Dihydro-2(1H)-quinolinone accumulated, but it was degraded further immediately and rapidly, when tested as sole substrate in the microcosms. This trend was also found analyzing the ratios $R_{2(1H)\text{-quinolinone}}$ and $R_{3,4\text{-dihydro-}2(1H)\text{-quinolinone}}$ at the Castrop-Rauxel and the Düsseldorf–Flingern site: $R_{2(1H)\text{-quinolinone}}$ reached higher values than $R_{3,4\text{-dihydro-}2(1H)\text{-quinolinone}}$ while this tendency was not found at the Wülknitz site. In all the degradation experiments degradation of isoquinoline in comparison to quinoline was much slower. The hydroxylation of isoquinoline occurred at a lower rate than observed for quinoline. These findings were in accordance to field data: higher distribution of isoquinoline all over the sites and larger plume thickness of isoquinoline in comparison to quinoline in the high-resolution well. At most tar oil contaminated sites the number of wells for the evaluation of groundwater is limited. Therefore, compound ratios should be proposed which give best indication for natural attenuation, even if only few samples are available to analyse:

At all the three sites investigated $R_{1(2H)\text{-isoquinolinone}}$ reached highest values, which were significantly higher than $R_{2(1H)\text{-quinolinone}}$ and $R_{3,4\text{-dihydro-}2(1H)\text{-quinolinone}}$. In a large number of groundwater samples from wells downstream the source, quinoline was below the limit of detection, while isoquinoline was still present. When discussing the best ratio for the evaluation of natural attenuation in the future, isoquinoline seems to be the better parent compound for the calculation of ratios. This conclusion takes into account that only those ratios were formed, where both compounds, hydroxylated quinoline compound as well as parent compound, were present in the groundwater. When including those samples, where no quinoline is detectable, by using the limit of detection of quinoline for the calculation of ratios, even higher values of ratio are reached for the two quinoline couples and makes the quinoline couples also highly useful as indication of natural attenuation. Also the ratios of methylsubstituted analogs were found to be feasible, while the $R_{3,4\text{-dihydro-}1(2H)\text{-isoquinolinone}}$ is found of minor value.

In best-case studies as they are present here, where a high number of wells widely distributed over the field sites was available, additionally the following two considerations are indicative for natural attenuation:

- The physicochemical properties of the isomeric couples 2(1H)-quinolinone/quinoline and 1(2H)-isoquinolinone/isoquinoline are almost identical, therefore the ratios have to be similar distributed if biodegradation is absent. Any variation, for instance a decrease in $R_{2(1H)\text{-quinolinone}}$ and an increase in $R_{1(2H)\text{-isoquinolinone}}$, shows differences in biodegradability and indicates the presence of natural attenuation.
- The variation in values of one compounds ratio, e.g. $R_{2(1H)\text{-quinolinone}}$, over the field site clearly shows differences in microbial activity at the various locations of the site and indicates the presence of natural attenuation, too.

These considerations show that at tar oil contaminated sites with a high number of wells various approaches will allow the identification of natural attenuation. The ratio approach, however, might be a simple and rapid procedure to detect natural attenuation at those sites, where the number of wells is restricted.

The described ratio approach of quinoline compounds is an important piece of a puzzle to evaluate natural attenuation as a cost-effective method in the groundwater at tar oil contaminated sites.

5. GENERAL CONCLUSION OF THE THESIS

Besides other strategies natural attenuation might reduce the risks posed by subsurface contamination. Natural attenuation is a passive approach relying on the ability of the intrinsic microorganisms to degrade contaminants. Demonstration, verification and monitoring of the on-going biodegradation is a necessity.

Implications for natural attenuation were obtained here from general site-directed information. Beneath the identification of the presence of anaerobic redox-conditions and reduced electron acceptors, the size of the plumes, especially at the Castrop-Rauxel site, implicated the presence of natural attenuation. Taking into account the groundwater velocity and time of existence of the contamination as the influencing parameters, a plume size is expected in Castrop-Rauxel, which is twice as large as the plume observed today.

The following approaches of this thesis revealed the presence of natural attenuation at the sites for the group of NSO-heterocyclic compounds:

Mass balances

The site survey allowed the long distance comparison of the concentration of various compounds. An optimum situation exists at the site of Castrop-Rauxel for the calculation of mass balances, since a well-structured field site is available with a high number of wells in the in- and out-flow, which are directly behind each other in the groundwater flow direction. The wells at the Düsseldorf-Flingern site are far from laying in line in the flow direction and the Wülknitz site is much more restricted for the calculation of mass loads, because of the limited number of wells, distributed over high distances and, furthermore, known changes in groundwater flow direction.

In groundwater of the three sites indications of specific compounds degradation was obtained from the relative change of concentration of compounds with similar physicochemical properties along the flow line. High differences in decrease of contaminants concentration were found: while the group of N-heterocycles was generally dominating the compounds mixture in the highest contaminated region, the group of S- and O-heterocycles take over the role downstream the source, while methylsubstituted compounds remained to be of high relevance. A comparison of heterocycles and EPA-PAHs indicated the importance to include heterocycles in routine analytics of tar oil contaminated sites. Especially acenaphthene is known as one of the most persistent PAHs. The presented data indicate that the group of S- and O-heterocycles gained the same or even higher relevance downstream the source.

Microcosms and on-site columns

Microcosms were performed by using groundwater with the authentic contamination (from the Düsseldorf-Flingern and Wülknitz site). In addition, the microbial activity in an on-site column filled with aquifer material and contaminated groundwater from the Wülknitz field site was analysed. The influence of variation of redox conditions as well as addition of reducing agents was studied and found of high relevance. Highest degradative potential was seen under sulfate-reducing conditions, the prevailing conditions at the field sites.

A broad number of heterocycles was found to be degradable. The degradation of contaminants and accumulation of metabolites demonstrated the presence of adapted microorganisms in the aquifer materials from the sites. Higher degradability of quinoline compounds in comparison to S- and O-heterocycles was found in accordance to compounds relevance downstream the field sites.

Plume fringe

The high-resolution well at the Düsseldorf-Flingern site allowed the micro-distance comparison of the concentration of various contaminants as well as electron acceptors across the fringe and centre zones and therefore a comparison of different contaminant plumes. The presence of natural attenuation was seen at the fringe of the plume by the correlation of decrease in concentration of especially sulfate and contaminants. Differences in the vertical plumes size for compounds with similar physicochemical properties and similar concentration levels were used as indications for differences in biodegradability of heterocycles and homocycles. Therefore, differences in degradability of isomers were worked out. Methylsubstituted heterocyclic 2-ring compounds showed a broader plume size suggesting less degradability compared to their non-substituted analogs.

Furthermore, the decrease of compounds such as quinoline and isoquinoline and accumulation of metabolites 2(1H)-quinolinone and 1(2H)-isoquinolinone at the fringe indicated a better correlation than studying the situation in the field using conventional multilevel wells with long filter units.

Metabolite/parent ratio approach

The presence of hydroxylated quinoline and isoquinoline compounds can be used as indicator for natural attenuation. However, the analyses of seven tar products showed that hydroxylated compounds, 2(1H)-quinolinone, 3,4-dihydro-2(1H)-quinolinone, 1(2H)-isoquinolinone and 3,4-dihydro-1(2H)-isoquinolinone are also primary constituents. Their detection in groundwater as a stand-alone indicator is found to be non-sufficient to demonstrate the occurrence of biological natural attenuation processes.

Instead, the ratio of hydroxylated to parent compound ($R_{metabolite}$) was verified as a useful indicator. Non-destructive processes such as dilution, volatilization, sorption and dissolution from tar oils influencing the distribution and fate of parent and hydroxylated quinoline compounds were analysed: Dilution and volatilization did not influence the $R_{metabolite}$. Dilution influenced both compound types in the same way and volatilization of these type of compounds is negligible. Sorption was found to influence both partners of the $R_{metabolite}$ at a minor extend. With respect to the partition coefficient between tar oil and water, the ratio in groundwater would be approximately 10 times higher than the highest ratio determined in tar oil. When paying attention to these parameters, about one third of groundwater samples exceed the highest tar oil ratio at all of the three sites investigated indicating the presence of natural attenuation.

Final remarks

The decrease in concentration along the plumes together with the results from the more artificial degradation experiments and the high-resolution well led to the general conclusion that biodegradative processes of heterocycles are present in the anaerobic aquifers of the three sites.

The variations in groundwater flow direction as well as heterogeneity of the sources of contaminant remain the most limiting factors for the assessment of natural attenuation capacity under field conditions. To understand and find biodegradative processes in the field sites, studies using small scale approaches are reasonable to find clear correlations. Generally, the number of wells at contaminated sites is often limited. Therefore, the approach using $R_{metabolite}$ of hydroxylated and parent quinoline compounds as an indicator may be a possibility to find indications for natural attenuation fast and with a limited number of samples. This

approach should be expanded to a higher number of compound couples, including studies of tar oils for the presence of other compounds, which are also discussed as metabolites.

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7. APPENDIX

7.1 Chemicals

Quinolines	CAS-Number	purity [%]	company
quinoline	CAS: [91-22-5]	98	Aldrich (241571)
isoquinoline	CAS: [119-65-3]	>97	Fluka (58740)
1-methylisoquinoline	CAS: [1721-93-3]	97	Aldrich (264938)
3-methylisoquinoline	CAS: [1125-80-0]	98	Aldrich (129895)
2-methylquinoline	CAS: [91-63-4]	≥98	Merck (8.05805)
4-methylquinoline	CAS: [491-35-0]	99	Aldrich (158283)
6-methylquinoline	CAS: [91-62-3]	98	Aldrich (108928)
2(1H)-quinolinone	CAS: [95-31-4]	99	Fluka (270873)
1(2H)-isoquinolinone	CAS: [491-30-5]	≥99	Fluka (55433)
4(1H)-quinolinone	CAS: [611-36-9]	98	Aldrich (H58005)
5(1H)-quinolinone	CAS: [578-67-6]	99	Aldrich (128791)
6-quinolinol	CAS: [580-16-5]	95	Aldrich (304484)
7-isoquinolinol	CAS: [7651-83-4]	97	Alfa Aesar (L17067)
3(2H)-isoquinolinone	CAS: [7651-81-2]	99	Aldrich (368954)
3,4-dihydro-2(1H)-quinolinone	CAS: [553-03-7]	98	Aldrich (415936)
3,4-dihydro-1(2H)-isoquinolinone	CAS: [1196-38-9]	>98	Pharm Lab (50-0056)
2-methyl-6-quinolinol	CAS: [613-21-8]	98	Alfa Aesar (A18846)
4-methyl-2(1H)-quinolinone	CAS: [607-66-9]	97	Aldrich (H43601)
6-methoxyquinoline	CAS: [5263-87-6]	98	Aldrich (183067)
1-methyl-2-quinolinone	CAS: [606-43-9]	99	Lancaster (9410)
2-methyl-4(1H)-quinolinone	CAS: [607-67-0]	98.5	Aldrich (H43806)
2,4-dimethylquinoline	CAS: [1198-37-4]	97	Aldrich (D184403)
2,6-dimethylquinoline	CAS: [877-43-0]	98	Aldrich (144029)
1,2,3,4-tetrahydroquinoline	CAS: [635-46-1]	98	Aldrich (T15504)
7-hydroxy-4-methyl-2(1H)-quinolinone	CAS: [20513-71-7]	≥97	Fluka (55627)
4-hydroxy-1-methyl-2(1H)-quinolinone	CAS: [1677-46-9]	98	Aldrich (168696)
Further N-Heterocycles			
pyrrol	CAS: [109-97-7]	98	Aldrich (131709)
pyridine	CAS: [110-86-1]	99.5	Merck (109728)
2-methylpyridine	CAS: [109-06-8]	≥98	Merck (809722)
3-methylpyridine	CAS: [108-99-6]	≥98	Merck (807048)
4-methylpyridine	CAS: [108-89-4]	≥98	Merck (807049)
indole	CAS: [120-72-9]	99	Aldrich (13408)
carbazole	CAS: [86-74-8]	98	Fluka (21790)
acridine	CAS: [260-94-6]	97	Aldrich (A23609)
9(10H)-acridinone	CAS: [578-95-0]	99	Fluka (01670)
6(5H)-phenanthridinone	CAS: [1015-89-0]	97	Aldrich (299634)
S-Heterocycles			
thiophene	CAS: [110-02-1]	≥99	Merck (8.08157)
2-methylthiophene	CAS: [554-14-3]	≥98	Aldrich (M84208)
3-methylthiophene	CAS: [616-44-4]	98	Fluka (69370)
2,5-dimethylthiophene	CAS: [638-02-8]	98.5	Aldrich (D188603)
tetrahydrothiophene	CAS: [110-01-0]	99	Aldrich (T15601)
benzothiophene	CAS: [95-15-8]	≥98	Merck (8.41538)
2-methylbenzothiophene	CAS: [1195-14-8]	99.4	Chiron (0946,9)
3-methylbenzothiophene	CAS: [1455-18-1]	99.4	Chiron (0947,9)
5-methylbenzothiophene	CAS: [14315-14-1]	99.5	Chiron (0356,9)
2,5-dimethylbenzothiophene	CAS: [16587-48-7]	99.9	Chiron (0954,10)
dibenzothiophene	CAS: [132-65-0]	98	Aldrich (D32202)
O-Heterocycles			
furan	CAS: [110-00-9]	99	Fluka (47990)
benzofuran	CAS: [271-89-6]	≥99	Fluka (28165)
2-methylbenzofuran	CAS: [4265-25-2]	96	Aldrich (224340)

	CAS-Number	purity [%]	company
2,3-dimethylbenzofuran	CAS: [3782-00-1]	97	Aldrich (457019)
dibenzofuran	CAS: [132-64-9]	≥99	Merck (8.20408)
4-methyldibenzofuran	CAS: [7320-53-8]	99.9	Chiron (1103.13)
BTEX			
benzene	CAS: [71-43-2]		BTEX mix, 2g/L in Methanol, Supelco (47993)
toluene	CAS: [108-88-3]		
o-xylene	CAS: [95-47-6]		
ethylbenzene	CAS: [100-41-4]		
p-xylene	CAS: [106-42-3]		
m-xylene	CAS: [108-38-3]		
PAHs/Homocycles			
naphthalene	CAS: [91-20-3]	99	Merck (8.20846)
1-methylnaphthalene	CAS: [90-12-0]	95	Aldrich (M56808)
2-methylnaphthalene	CAS: [91-57-6]	97	Aldrich (M57006)
1,3-dimethylnaphthalene	CAS: [575-41-7]	96	Aldrich (D170208)
acenaphthene	CAS: [83-32-9]	99	Sigma (215376)
fluorene	CAS: [86-73-7]	≥99	Fluka (46880)
indan	CAS: [496-11-7]	95	Aldrich (I1804)
indene	CAS: [95-13-6]	≥99	Aldrich (193828)
2-methylindene	CAS: [2177-47-1]	98	Aldrich (449431)
Internal standards			
toluene-D8	CAS: [2037-26-5]	100	Aldrich (233382)
naphthalene-D8	CAS: [1146-65-2]	99	Aldrich (176044)
furan-D4	CAS: [6142-90-1]	99.3	Ehrensdorfer C13965010)
pyridine-D5	CAS: [7291-22-7]	99.8	Ehrensdorfer (6646100)
2,5-thiophene-D2	CAS: [2041-41-1]	99	Ehrensdorfer (D-2859)
quinoline-D7	CAS: [34071-94-8]	98.6	Dr. Ehrensdorfer (MD-1450)

solvents	
acetone	LiChrosolv® for high performance liquid chromatography, Merck, (Darmstadt, Germany (1.00020.2500))
acetonitrile	Ultra Gradient HPLC Grade, J.T. Baker (9017) and Honeywell Speciality Chemicals (Seelze, Germany)
deuterium oxide (99.9 atom%)	Deutero GmbH, Kastellaun, Germany
dimethylsulfoxide Uvasol®	Merck, Darmstadt, Germany
dimethylsulfoxide-D_6 (100 atom%)	Eurisotop;Gif-sur-Yvette, France
deuterated chloroform (100 atom%)	Eurisotop; Gif-sur-Yvette, France
ethylacetate extra pure	Merck, Darmstadt, Germany
methanol	SupraSolv® für die Gaschromatographie, Merck (1.06011.2500) and LiChrosolv® for high performance liquid chromatography, Merck (1.06007.2500)
millipore water	Milli-Q purification system, Millipore, Eschborn, Germany
tetrahydrofurane LiChrosolv®	Merck, Darmstadt, Germany

7.2 Physicochemical data of investigated tar oil compounds

Table 22: Physico-chemical data of heterocyclic and homocyclic compounds.

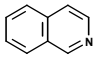
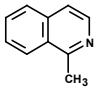
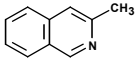
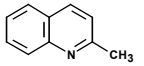
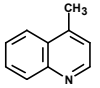
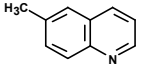
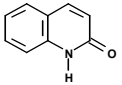
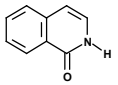
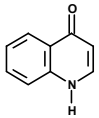
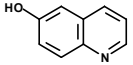
compound	structure	molecular weight [g/mol]	density[c] [g/cm^3]	log P_{OW}	water solubility [mg/L][d]	pKa[c]	Henry´s law constant [atm × m^3/mol][s]
N-Heterocycles							
quinoline		129.16	1.106	2.14 [b]	6191[d]	5.0 most basic	6.9×10^{-7}
isoquinoline		129.16	1.106	2.14 [b]	5663 [d]	5.4 most basic	6.9×10^{-7}
1-methylisoquinoline		143.19	1.076	2.69 [b]	409.6 [e]	6.2 most basic	7.6×10^{-7}
3-methylisoquinoline		143.19	1.076	2.68 [a]	417.6 [e]	5.7 most basic	7.6×10^{-7}
2-methylquinoline		143.19	1.076	2.69 [b]	498.5 [e]	5.9 most basic	7.6×10^{-7}
4-methylquinoline		143.19	1.085	2.69 [b]	479.2 [e]	5.7 most basic	7.6×10^{-7}
6-methylquinoline		143.19	1.076	2.57 [a]	631.1 [e]	5.2 most basic	7.6×10^{-7}
2(1H)-quinolinone		145.16	1.188	1.26 [a]	1371 [d]	-0.4 most basic 11.8 most acidic	6.6×10^{-10}
1(2H)-isoquinolinone		145.16	1.188	1.42 [b]	514 [d]	-1.2 most basic 13.6 most acidic	1.7×10^{-9}
4(1H)-quinolinone		145.16	1.188	1.66 [b]	4800 [f]	4.3 most basic 11.2 most acidic	7.2×10^{-11}
6-quinolinol		145.16	1.260	1.98 [a]	455 [f]	5.2 most basic 8.9 most acidic	7.2×10^{-11}

Table 22: continued (part 2).

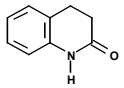
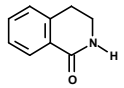
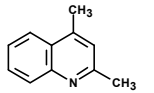
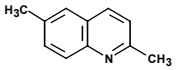
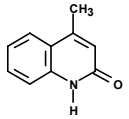
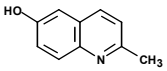
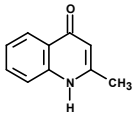
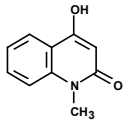
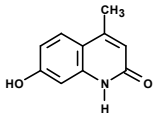
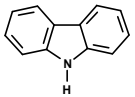
compound	structure	molecular weight [g/mol]	density[c] [g/cm^3]	log P_{OW}	water solubility [mg/L][d]	pKa[c]	Henry´s law constant [atm × m^3/mol][s]
3,4-dihydro-2(1H)-quinolinone		147.18	1.142	1.34 [p]	883 [d]	1.5 most basic 14.8 most acidic	3.0×10^{-9}
3,4-dihydro-1(2H)-isoquinolinone		147.18	1.142	1.34 [p]	37033 [d]	-1.5 most basic 14.6 most acidic	2.4×10^{-9}
2,4-dimethylquinoline		157.21	1.052	3.24 [b]	1800 [f]	6.5 most basic	8.4×10^{-7}
2,6-dimethylquinoline		157.21	1.052	3.24 [b]	1800 [f]	6.1 most basic	8.4×10^{-7}
4-methyl-2(1H)-quinolinone		159.19	1.136	1.87 [b]	1757 [e]	11.8 most acidic -0.4 most basic	1.0×10^{-9}
2-methyl-6-quinolinol		159.19	1.210	2.21 [b]	3404 [e]	9.1 most acidic 6.0 most basic	7.9×10^{-11}
2-methyl-4(1H)-quinolinone		159.19	1.210	1.84 [b]	1849 [e]	4.4 most acidic 11.9 most basic	4.4×10^{-9}
4-hydroxy-1-methyl-2(1H)-quinolinone		177.20	1.326	-1.29 [b]	715300 [e]	4.5 most acidic -0.3 most basic	1.6×10^{-19}
7-hydroxy-4-methyl-2(1H)-quinolinone		175.19	1.264	2.48 [b]	1685 [e]	9.70 most acidic -0.7 most basic	8.2×10^{-15}
carbazole		167.21	1.229	3.72 [a]	1.8 [g]	17.0 most acidic -0.3 most basic	8.7×10^{-8}

Table 22: continued (part 3).

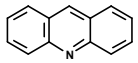
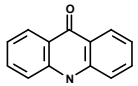
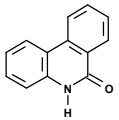
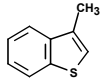
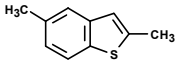
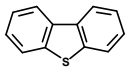
compound	structure	molecular weight [g/mol]	density[c] [g/cm^3]	log P_{OW}	water solubility [mg/L][d]	pKa[c]	Henry´s law constant [atm × m^3/mol][s]
acridine		179.22	1.187	3.40 [a]	38.4 [h]	5.5 most basic	6.7×10^{-8}
9(10H)-acridinone		195.22	1.230	2.84 [b]	13.53 [e]	-0.3 most basic	7.0×10^{-12}
6(5H)-phenanthridinone		195.22	1.230	1.30 [b]	278.7 [e]	13.3 most acidic -1.5 most basic	4.3×10^{-10}
pyrrol		67.09	0.990	0.75 [a]	45000 [j]	17.0 most acidic -0.3 most basic	9.1×10^{-6}
pyridine		79.10	0.956	0.65 [a]	729800 [e]	5.2 most basic	7.1×10^{-6}
2-methylpyridine		93.13	0.941	1.11[a]	661.6 [e]	6.0 most basic	7.8×10^{-6}
3-methylpyridine		93.13	0.941	1.20 [a]	661.6 [e]	5.5 most basic	7.8×10^{-6}
4-methylpyridine		93.13	0.941	1.22 [a]	661.6 [e]	5.9 most basic	7.8×10^{-6}
S-Heterocycles							
benzothiophene		134.20	1.187	3.12 [a]	130 [l]	-	2.9×10^{-4}
3-methylbenzothiophene		148.22	1.146	3.54 [b]	73.74 [e]	-	3.2×10^{-4}
2,5-dimethylbenzothiophene		162.25	1.114	4.08 [b]	21.8 [e]	-	3.5×10^{-4}
dibenzothiophene		184.26	1.252	4.38 [b]	1.47 [i]	-	2.8×10^{-5}

Table 22: continued (part 4).

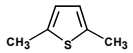
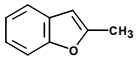
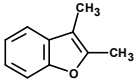
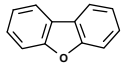
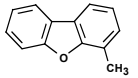
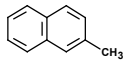
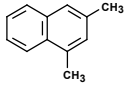
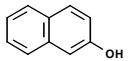
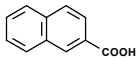
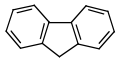
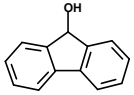
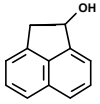
compound	structure	molecular weight [g/mol]	density[c] [g/cm^3]	log P_{OW}	water solubility [mg/L]	pKa[c]	Henry´s law constant [atm × m^3/mol][s]
thiophene		84.14	1.066	1.81 [a]	3010 [f]	-	2.9×10^{-3}
2-methylthiophene		98.16	1.031	2.33 [a]	1212 [e]	-	3.2×10^{-3}
3-methylthiophene		98.16	1.031	2.34 [a]	400 [m]	-	3.2×10^{-3}
2,5-dimethylthiophene		112.19	1.006	2.91 [b]	351.5 [e]	-	3.6×10^{-3}
O-Heterocycles							
benzofuran		118.13	1.110	2.67 [a]	534.8 [e]	-	5.3×10^{-4}
2-methylbenzofuran		132.16	1.077	3.22 [p]	160.4 [e]	-	5.8×10^{-4}
2,3-dimethylbenzofuran		146.19	1.052	3.63 [b]	62.2 [e]	-	6.4×10^{-4}
dibenzofuran		168.19	1.197	4.12 [a]	3.1 [n]	-	5.1×10^{-5}
4-methyldibenzofuran		182.22	1.162	4.60 [b]	0.5 [e]	-	4.3×10^{-5}
furan		68.08	0.942	1.34 [a]	1000 [o]	-	5.4×10^{-3}
Homocycles/PAHs							
naphthalene		128.16	1.037	3.30 [a]	31 [l]	-	5.3×10^{-4}
1-methylnaphthalene		142.2	1.016	3.87 [a]	40.62 [f]	-	5.8×10^{-4}
2-methylnaphthalene		142.2	1.016	3.86 [a]	24.6 [f]	-	5.8×10^{-4}
1,3-dimethylnaphthalene		156.22	1.000	4.42 [a]	8 [f]	-	6.4×10^{-5}

Table 22: continued (part 5).

compound	structure	molecular weight [g/mol]	density[c] [g/cm^3]	log P_{OW}	water solubility [mg/L]	pKa[c]	Henry´s law constant [atm × m^3/mol][s]
1-naphthol		144.17	1.181	2.85 [a]	866 [i]	-	5.5×10^{-8}
2-naphthol		144.17	1.181	2.70 [a]	755 [f]	-	5.5×10^{-8}
2-naphthoic acid		172.18	1.265	3.28 [a]	47 [m]	4.2 most acidic	1.0×10^{-8}
indan		118.18	0.997	3.18 [a]	109 [f]	-	3.8×10^{-3}
indene		116.16	1.038	2.92 [a]	332.4	-	1.6×10^{-3}
1-indanone		132.16	1.148	2.11 [b]	1427 [e]	-	4.8×10^{-6}
fluorene		166.22	1.120	4.18 [a]	1.89 [q]	-	1.7×10^{-4}
9-fluorenol		182.22	1.253	2.71 [b]	65.4 [e]	13.3 most acidic	6.2×10^{-9}
acenaphthene		154.21	1.143	3.92 [a]	3.9 [r]	-	2.8×10^{-4}
1-acenaphthenol		170.21	1.290	2.61 [b]	90.5 [e]	13.7 most acidic	1.0×10^{-8}

[a]Hansch et al. (1995); [b]U.S. EPA. 2000. Episuite KOWIN-Program; [c]SciFinder (1994-2006); [d]determined in this study by HPLC-DAD or HPLC-MS-MS; [e]U.S. EPA. 2000. Episuite WSKOW-Program; [f]Yalkowsky and Dannenfelser (1992); [g]Ainsworth et al. (1989); [h]Banwart et al. (1982); [i] Hassett et al. (1980); [j]Riddick et al. (1986); [k]Goe (1978); [l] Pearlman et al. (1984); [m] Chem Inspect Test Inst (1992); [n] Lu et al. (1978); Valvani et al. (1981); [p] Sangster (1994); [q]Wauchope and Getzen (1972); [r]Miller et al. (1985); [s] U.S. EPA (2000). Episuite Henry Program v 3.10: bond contribution.

7.3 Analytics of heterocyclic compounds

Table 23: Limits of detection (LOD) of heterocyclic and homocyclic compounds in the HPLC-DAD method.

compound	LOD [µg/L]
carbazole	0.4
6(5H)-phenanthridinone	0.4
acridine	2.4
9(10H)-acridinone	2.4
benzothiophene	0.8
3-methylbenzothiophene	0.8
2,5-dimethylbenzothiophene	0.8
dibenzothiophene	0.8
benzofuran	0.4
2-methylbenzofuran	0.4
2,3-dimethylbenzofuran	0.4
dibenzofuran	0.4
4-methyldibenzofuran	0.4
naphthalene	0.2
1-methylnaphthalene	0.4
2-methylnaphthalene	0.4
1,3-dimethylnaphthalene	0.4
2-naphthol	0.4
1-naphthol	0.4
2-naphthoic acid	1.2
fluorene	0.6
9-fluorenol	0.4
acenaphthene	1.2
1-acenaphthenol	0.2
indene	0.4
indan	1.6
1-indanone	0.4

(according to Mundt and Hollender, 2005).

Method development: HPLC-MS-MS

Table 24: Calibration curves of quinoline compounds without and within matrix as well as the linear correlation coefficient (R^2).

compound	standards without matrix		standards in matrix	
	calibration curve	R^2	calibration curve	R^2
quinoline	y = 7393x + 6843	0.999	y = 7676x + 35939	1.000
isoquinoline	y = 11323x + 11288	0.999	y = 11212x + 110644	1.000
1-methylisoquinoline	y = 49200x + 427266	0.998	y = 44497x + 314883	0.999
3-methylisoquinoline	y = 28827x + 84398	0.997	y = 28328x + 205289	1.000
2-methylquinoline	y = 7679x - 10062	1.000	y = 7530x + 8302	1.000
4-methylquinoline	y = 17918x - 151	1.000	y = 17710x + 24437	0.999
6-methylquinoline	y = 22227x + 109869	0.999	y = 22016x + 95136	0.999
2(1H)-quinolinone	y = 24941x + 17505	0.999	y = 25822x + 40278	1.000
1(2H)-isoquinolinone	y = 3512x + 4115	0.999	y = 3530x + 5921	0.999
4(1H)-quinolinone	y = 4706x + 28039	0.999	y = 4556x + 35924	0.999
5(1H)-quinolinone	y = 3757x + 10628	1.000	y = 3698x + 38462	0.997
6-quinolinol	y = 6285x + 17856	1.000	y = 5976x + 35600	0.999
7-isoquinolinol	y = 7521x + 76848	0.996	y = 7420x + 17943	1.000
3(2H)-isoquinolinone	y = 9941x - 22470	0.999	y = 9703x - 24672	0.999
3,4-dihydro-2(1H)-quinolinone	y = 5385x + 18855	0.998	y = 5675x - 11771	1.000
3,4-dihydro-1(2H)-isoquinolinone	y = 16504x + 5569	1.000	y = 16400x + 19860	1.000
2-methyl-6-quinolinol	y = 7210x + 26000	0.999	y = 6700x + 53390	0.998
4-methyl-2(1H)-quinolinone	y = 17106x - 22742	0.999	y = 17130x + 15584	1.000
6-methoxyquinoline	y = 67238x + 641068	0.998	y = 66775x + 718946	0.998
1-methyl-2-quinolinone	y = 14132x + 3538	1.000	y = 14304x + 25683	1.000
4-hydroxy-1-methyl-2(1H)-quinolinone	y = 7209.6x + 26000	0.999	y = 6700x + 53390	0.998
2,4-dimethylquinoline	y = 41869x + 146198	0.999	y = 37924x + 90239	0.999
2,6-dimethylquinoline	y = 48608x + 320287	0.999	y = 48473x + 62614	0.999
1,2,3,4-tetrahydroquinoline	y = 4992x - 153817	0.996	y = 3900x - 100280	0.989
4-methyl-2,7-quinolinol	y = 4827x + 17189	0.998	y = 4844x + 24022	1.000
1-methyl-2,4-quinolinol	y = 3609x + 26313	0.999	y = 3744x - 40639	0.994
D_7-quinoline	y = 3205x - 9547	1.000	y = 3187x + 4529	0.999

Method development: Headspace-GC-MS

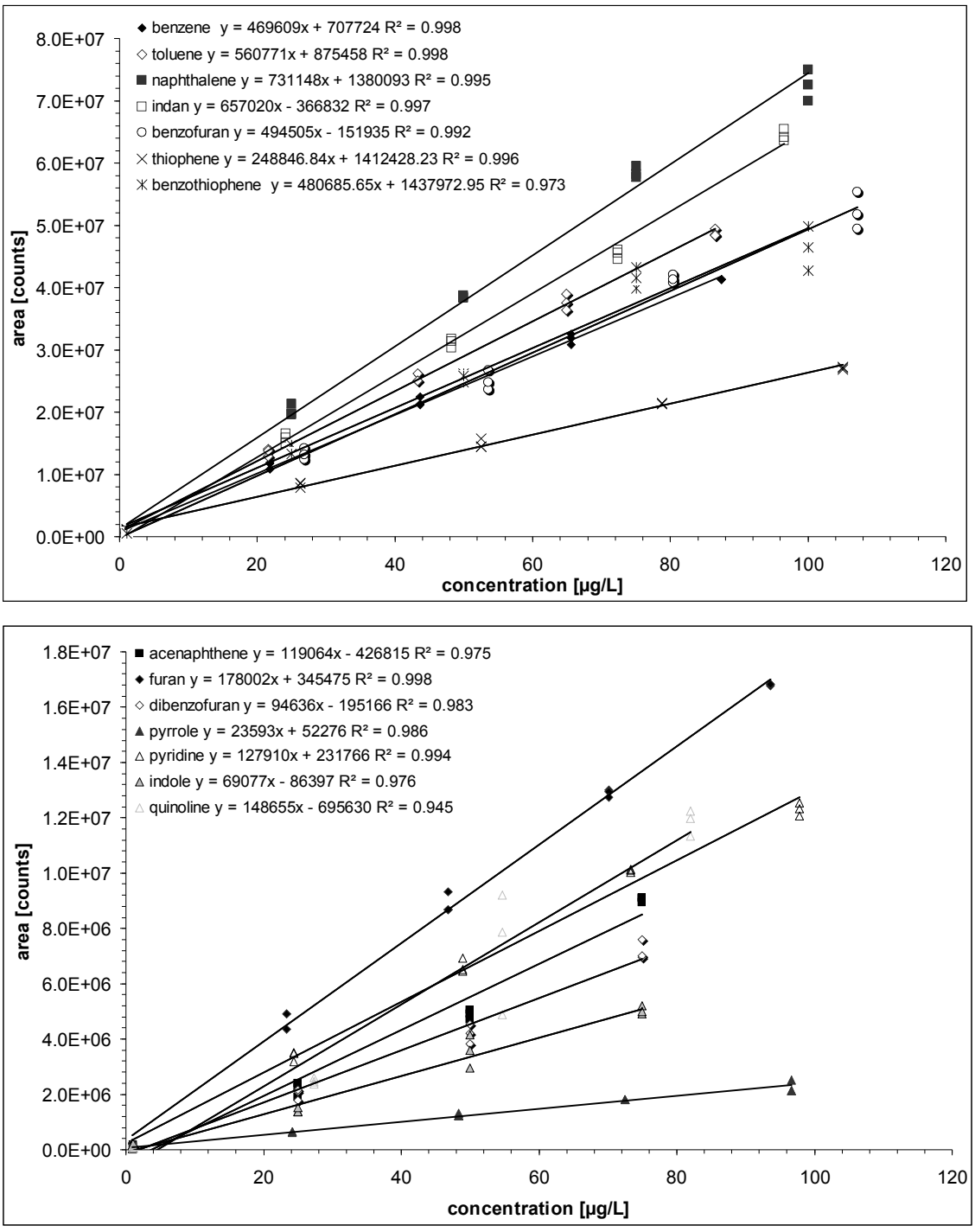


Figure 47: Concentration range characterized by linearity for selected heterocycles and homocylcles using the SIM-mode.

Application of the Headspace-GC-MS method to field measurements

Table 25: Monocyclic aromatic compounds determined by Headspace-GC of the Castrop-Rauxel, Wülknitz and Düsseldorf-Flingern field site. Concentration [µg/L].

	Castrop-Rauxel					Wülknitz						Düsseldorf-Flingern								
well	B6	B7	B220	B231	T18	12/98	20/05	13/01	22/01	06/98	14/01	19201 8 m	19201 10 m	19202 8 m	19202 10 m	19185 8 m	19185 10 m	19209 8 m	19209 10 m	19071
benzene	39	29	22	25	55	<0.8	4.2	2.5	3.5	0.9	<0.8	17	13	1.3	<0.8	27	1.2	38	3.5	1.6
toluene	71	26	8.3	21	<0.4	<0.4	17	1.0	0.7	<0.4	<0.4	3.7	848.9	<0.4	<0.4	13.8	1.3	6.6	<0.4	<0.4
o-xylene	23	2.7	1.7	2.4	<0.7	<0.7	9.4	2.0	0.9	1.1	<0.7	0.8	72.7	<0.7	<0.7	14.0	<0.7	352	<0.7	<0.7
furan	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4
pyrrol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pyridine	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7	<2.7
2-methylpyridine	576	<2.4	<2.4	<2.4	<2.4	<2.4	4.8	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
3-methylpyridine	94	<2.4	<2.4	<2.4	<2.4	<2.4	11.0	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
4-methylpyridine	58	<2.4	<2.4	<2.4	<2.4	<2.4	43.8	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
thiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
2-methylthiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
3-methylthiophene	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7
dimethylthiophene	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6
tetrahydrothiophene	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9	<0.9

LOD of pyrrol was not determined.

7.4 Site-directed analyses: Relevance of heterocyclic compounds

7.4.1 Castrop-Rauxel: Field data

Castrop-Rauxel: HPLC-DAD data

Table 26: HPLC-DAD data from the Castrop-Rauxel field site; sampling March 2005 (part 1). Concentration [µg/L].

compound	T11			T12			T13			T14			T15		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
carbazole	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
6(5H)-phenanthridinone	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
acridine	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
9(10H)-acridinone	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
benzothiophene	1.6	140	418	72.3	372	671	890	1274	1348	1062	1228	5459	158	1012	1284
3-methylbenzothiophene	<0.8	52	232	37	144	179	284	309	260	135	338	702	55	317	519
dibenzothiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
benzofuran	5.9	293	737	272	910	1157	965	1504	1242	1915	2508	1659	36	205	245
2-methylbenzofuran	<0.4	173	605	68	229	368	558	683	750	643	587	439	95	453	538
methylbenzofuran isomer 1	<0.4	83	312	41	141	168	245	268	283	230	304	187	64	200	263
2,3-dimethylbenzofuran	0.5	34	90	8.4	76	56	101	126	78	69	83	89	46	144	239
dibenzofuran	2.6	41.5	51.3	44.3	87.9	<0.4	<0.4	<0.4	89.2	84.7	<0.4	223.2	<0.4	193.0	235.6
naphthalene	36	656	3064	469	2844	4264	4149	4854	4901	3915	4395	4606	325	1880	3049
1-methylnaphthalene	7.3	137	448	188	735	636	847	914	783	632	677	747	74	1291	1763
2-methylnaphthalene	2.3	8.8	141	6.4	201	811	883	1344	1305	363	1024	1380	8.6	12	34
1,3-dimethylnaphthalene	<0.4	6.9	38	16	53	56	32	74	59	44	64	<0.4	11	130	214
2-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	<0.4	14.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	115	157
2-naphthoic acid	<1.2	309	<1.2	139	155	<1.2	177	218	222	366	<1.2	351	<1.2	<1.2	<1.2
fluorene	<0.6	<0.6	<0.6	8.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	7.8	86	160
acenaphthene	1.5	31	<1.2	31	203	109	229	37	257	126	208	182	25	309	389
acenaphthenol	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
indene	36	1504	3799	851	2688	2997	4212	5010	4989	3661	6656	619	573	1944	2125
indan	19	263	806	160	539	672	941	1063	964	868.	1073	960	152	968	1373
1-indanone	<0.4	278	505	11.7	372	532	813	960	775	507	465	432	32	593	816

n.a. = not analysed

Table 26, continued (part 2). Concentration [µg/L].

compound	T6			T7			T8			T9			T10		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
carbazole	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	311	1311	1556
6(5H)-phenanthridinone	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
acridine	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
9(10H)-acridinone	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
benzothiophene	430	462	788	446	412	518	649	788	1082	1118	926	1371	567	1241	1012
3-methylbenzothiophene	77	64	95	111	34	150	135	251	262	297	256	368	459	361	414
dibenzothiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
benzofuran	203	194	190	677	893	959	1297	1154	1117	1598	1098	1750	33	210	171
2-methylbenzofuran	365	462	509	351	335	545	724	692	807	686	420	1088	238	559	445
methylbenzofuran isomer 1	193	340	282	203	204	286	378	492	431	527	384	566	211	491	550
2,3-dimethylbenzofuran	78	53	95	83	84	99	135	143	93	136	1401	199	105	205	268
dibenzofuran	58	63	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	119.5	246	218
naphthalene	891	883	604	2422	2356	3076	4525	5336	5866	6045	5044	6766	1184	4686	1728
1-methylnaphthalene	155	176	424	446	317	468	330	570	640	829	660	1130	428	880	1059
2-methylnaphthalene	12	18	12	31	63	206	331	765	253	412	269	228	20	48	46
1,3-dimethylnaphthalene	23	32	15	25	18	<0.4	<0.4	<0.4	<0.4	86	97	131	39	157	137
2-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	56	43	45	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	19	7.9	<0.4	4.2	35	19
2-naphthoic acid	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	800	1424
fluorene	26	29	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	58	103	97
acenaphthene	78	80	44	14	19	<1.2	<1.2	<1.2	100	207	144	340	132	309	316
acenaphthenol	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
indene	2393	2408	1943	2153	2137	2919	4317	4225	4501	5830	4852	7355	901	3163	2094
indan	691	714	1171	641	643	702	665	853	1154	<1.6	760	<1.6	486	1170	1276
1-indanone	<0.4	<0.4	<0.4	<0.4	<0.4	413	1114	830	443	364	364	576	<0.4	134	<0.4

n.a. = not analysed

Table 26, continued (part 3). Concentration [µg/L].

compound	T1			T2			T3			T4			T5		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
carbazole	<0.4	<0.4	<0.4	<0.4	n.a.	<0.4	<0.4	<0.4	<0.4	<0.4	3.2	<0.4	<0.4	3.4	292
6(5H)-phenanthridinone	<0.4	<0.4	<0.4	<0.4	n.a.	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
acridine	<2.4	<2.4	<2.4	<2.4	n.a.	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
9(10H)-acridinone	<2.4	<2.4	<2.4	<2.4	n.a.	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
benzothiophene	17	25	379	10	n.a.	45	1.3	7.9	348	1.2	63	424	0.7	74	975
3-methylbenzothiophene	12	34	96	16	n.a.	14	3.4	4.9	65	7.2	44	111	<0.8	20	281
dibenzothiophene	<0.8	<0.8	<0.8	<0.8	n.a.	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
benzofuran	3.6	<0.4	125	9.2	n.a.	38	1.9	6.7	361	<0.4	25	27	<0.4	<0.4	81
2-methylbenzofuran	28	49	213	10	n.a.	28	12	15	245	7.3	32	131	<0.4	37	309
methylbenzofuran isomer 1	14	26	125	32	n.a.	19	7.9	9.3	109	5.6	29	169	<0.4	21	198
2,3-dimethylbenzofuran	6.7	4.3	42	10	n.a.	7.6	3.6	3.7	43	3.0	22	95	<0.4	6.3	101
dibenzofuran	4.9	3.9	60	<0.4	n.a.	<0.4	<0.4	<0.4	<0.4	<0.4	6.2	<0.4	<0.4	14	9.7
naphthalene	11	13	168	32	n.a.	73	12	38	535	16	132	125.	29	355	3277
1-methylnaphthalene	3.0	6.4	246	6.5	n.a.	17	1.3	5.9	194	5.5	33	21	6.3	53	573
2-methylnaphthalene	1.3	0.8	19	2.5	n.a.	1.8	0.4	2.4	61	1.2	0.8	<0.4	<0.4	0.8	103
1,3-dimethylnaphthalene	<0.4	<0.4	54	<0.4	n.a.	<0.4	<0.4	<0.4	8.4	<0.4	9.5	5.8	<0.4	7.0	3.7
2-naphthol	<0.4	<0.4	<0.4	<0.4	n.a.	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	<0.4	<0.4	<0.4	<0.4	n.a.	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthoic acid	<1.2	<1.2	<1.2	<1.2	n.a.	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
fluorene	1.0	0.9	21	<0.6	n.a.	<0.6	<0.6	<0.6	<0.6	<0.6	3.6	<0.6	<0.6	7.4	39
acenaphthene	4.5	5.3	63	6.0	n.a.	3.0	<1.2	1.2	31	1.2	37	74	3.0	25	165
acenaphthenol	<0.2	<0.2	<0.2	<0.2	n.a.	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
indene	3.9	3.8	1378	25	n.a.	80	6.5	20.3	1346	3.1	145	606	1.7	125	1730
indan	<1.6	25	401	29	n.a.	37	4.2	12	382	<1.6	50	540	10	70	868
1-indanone	<0.4	<0.4	<0.4	<0.4	n.a.	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

n.a. = not analysed

Castrop-Rauxel: HPLC-MS-MS data

Table 27: HPLC-MS-MS data from the Castrop-Rauxel field site; sampling March 2005 (part 1). Concentration [µg/L].

compound	T11			T12			T13			T14			T15		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
quinoline	0.028	5.13	3.07	0.490	6.63	9.17	126	75.6	59.9	257	283	458	0.830	40.4	235
isoquinoline	0.285	3.36	2.24	0.989	1.68	0.981	10.2	7.61	7.99	31.4	13.6	25.4	1.72	5.06	17.7
1-methylisoquinoline	0.015	0.870	0.355	0.068	0.532	0.682	16.4	9.44	9.19	33.9	20.3	36.6	0.155	6.11	26.7
3-methylisoquinoline	0.032	0.738	0.353	0.065	0.476	0.382	11.0	5.27	6.24	22.3	13.9	23.0	0.173	2.45	17.7
2-methylquinoline	0.012	5.39	2.72	0.365	4.11	5.31	124	77.9	57.4	266	150	298	0.944	37.9	251
4-methylquinoline	0.004	10.9	5.061	0.668	9.40	10.3	247	139	123	496	320	610	1.73	86.4	458
6-methylquinoline	0.006	0.900	0.389	0.059	0.681	0.712	15.3	8.30	7.42	29.3	37.2	46.6	0.128	3.95	24.3
2(1H)-quinolinone	0.032	39.9	101	5.50	469	370	3549	3414	2430	3337	3028	5613	8.43	1261	4050
1(2H)-isoquinolinone	0.386	20.4	15.7	16.1	41.0	24.8	371	290	159	719	557	850	265	3402	3060
4(1H)-quinolinone	0.027	0.989	0.691	0.125	0.349	<0.008	9.20	4.72	13.9	23.9	17.9	26.8	<0.008	4.98	17.9
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	14.4	8.32	12.3	8.60	15.0	9.87	<0.004	12.5	26.0
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	14.4	<0.004	<0.004	27.1	12.9	31.2	<0.004	<0.004	22.9
3,4-dihydro-2(1H)-quinolinone	<0.008	8.02	14.1	1.66	26.6	4.53	915	845	473	818	1194	2259	2.69	373	1670
3,4-dihydro-1(2H)-isoquinolinone	0.019	0.813	<0.006	0.160	0.615	0.513	15.2	9.96	14.6	33.2	17.2	36.6	<0.006	9.54	32.7
2-methyl-6-quinolinol	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	12.1	6.62	19.8	31.2	20.6	39.7	<0.003	5.34	30.2
4-methyl-2(1H)-quinolinone	0.008	5.88	8.61	8.78	25.0	4.92	296	305	185	426	414	443	9.89	718	916
6-methoxyquinoline	<0.003	0.625	0.230	0.032	0.338	0.406	9.61	5.43	6.02	19.6	12.7	23.2	0.085	3.61	19.9
1-methyl-2-quinolinone	0.047	1.12	0.467	0.191	0.812	0.682	16.1	8.23	13.4	32.8	19.2	32.8	2.41	13.5	51.0
2-methyl-4(1H)-quinolinone	0.091	2.02	2.267	0.339	0.863	0.619	8.10	5.23	12.9	18.5	12.5	20.0	1.23	7.36	14.9
2,4-dimethylquinoline	0.030	12.1	5.73	0.686	9.28	10.9	247	134	129	499	280	548	1.78	85.0	452
2,6-dimethylquinoline	0.017	0.893	0.464	0.055	0.642	0.846	17.0	10.1	9.80	38.5	19.7	38.5	0.163	4.28	28.6
1,2,3,4-tetrahydroquinoline	<0.013	<0.013	<0.013	<0.013	<0.013	0.367	4.59	<0.013	<0.013	14.4	7.92	13.4	<0.013	<0.013	11.5
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.005	1.02	<0.005	<0.005	0.282	0.37	9.90	5.32	11.4	23.4	13.4	<0.005	<0.005	<0.005	22.4
4-hydroxy-1-methyl-2(1H)-quinolinone	0.053	0.520	<0.006	0.077	<0.006	<0.006	5.63	3.54	<0.006	8.53	6.53	9.35	0.368	3.26	9.97

n.a. = not analysed

Table 27, continued (part 2). Concentration [µg/L].

compound	T6			T7			T8			T9			T10		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
quinoline	0.580	0.877	0.721	1.22	8.59	3.86	53.1	40.4	39.5	30.3	28.4	30.9	4.35	90.8	4.04
isoquinoline	17.0	10.4	8.83	4.46	35.5	6.44	13.5	6.29	5.24	8.09	2.94	4.54	1.98	8.34	5.28
1-methylisoquinoline	0.157	0.190	0.220	0.24	1.36	0.580	13.4	5.97	6.02	3.89	4.08	4.31	0.658	11.7	0.881
3-methylisoquinoline	<0.004	<0.004	<0.004	<0.004	<0.004	0.562	11.4	3.62	3.78	3.46	3.09	2.71	0.576	8.42	0.833
2-methylquinoline	0.384	0.319	0.815	1.29	7.89	3.78	57.5	42.7	44.0	30.0	29.7	26.2	4.90	96.6	4.90
4-methylquinoline	0.630	0.834	1.37	2.37	15.3	7.09	110	91.2	77.3	54.7	58.0	53.4	9.67	194	9.67
6-methylquinoline	0.207	<0.004	0.271	0.154	1.32	0.59	12.3	3.78	4.46	3.74	3.94	2.96	0.518	10.7	0.65
2(1H)-quinolinone	4.46	3.91	6.95	8.89	79.0	292	1656	5222	2547	293	45.0	3859	2.04	30.0	4.47
1(2H)-isoquinolinone	38.6	39.7	80.2	<0.034	<0.034	19.7	329	354	178	936	903	626	252	911	558
4(1H)-quinolinone	0.685	0.831	2.34	0.223	1.47	0.659	<0.008	<0.008	4.34	<0.008	3.59	<0.008	<0.008	9.32	<0.008
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	9.92	<0.004	<0.004	<0.004	7.16	<0.004	1.18	2.61	<0.004
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3,4-dihydro-2(1H)-quinolinone	<0.008	<0.008	<0.008	2.06	17.9	9.75	46.3	1058	660	18.6	10.6	1196	<0.008	12.9	4.18
3,4-dihydro-1(2H)-isoquinolinone	<0.006	<0.006	<0.006	0.253	0.725	<0.006	16.8	6.21	1.98	<0.006	<0.006	<0.006	<0.006	14.6	5.87
2-methyl-6-quinolinol	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	13.9	15.5	17.8	10.1	88.0	6.56	449	287	204	774	498	669	63.3	281	94.2
6-methoxyquinoline	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
1-methyl-2-quinolinone	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	3.927	5.890	2.337	13.387	2.256
2-methyl-4(1H)-quinolinone	2.52	2.88	4.25	0.86	6.43	1.12	36.1	7.34	6.68	<0.006	6.98	6.28	3.09	13.1	4.91
2,4-dimethylquinoline	1.79	0.981	5.62	2.35	15.1	7.05	110	87.2	76.3	50.9	54.7	47.3	8.91	176	9.04
2,6-dimethylquinoline	<0.006	0.073	0.264	0.123	0.906	0.589	13.6	6.33	5.47	4.97	3.31	4.29	0.464	11.4	0.561
1,2,3,4-tetrahydroquinoline	<0.013	1.04	1.39	<0.013	1.32	<0.013	6.10	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.005	<0.005	<0.005	0.200	1.71	0.407	17.6	3.33	0.822	<0.005	<0.005	<0.005	<0.005	8.45	2.32
4-hydroxy-1-methyl-2(1H)-quinolinone	<0.006	<0.006	0.307	0.096	0.792	<0.006	8.52	3.25	2.74	1.90	2.29	3.58	0.828	2.84	1.26

n.a. = not analysed

Table 27, continued (part 3). Concentration [µg/L].

compound	T1			T2			T3			T4			T5		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
quinoline	0.022	0.017	0.321	0.156	n.a	0.313	0.083	<0.006	<0.006	0.069	0.073	<0.006	0.065	0.052	<0.006
isoquinoline	0.058	0.038	0.085	0.071	n.a	0.167	0.019	0.009	0.098	0.037	0.093	0.206	0.009	0.078	1.13
1-methylisoquinoline	0.020	0.017	0.054	0.027	n.a	0.044	0.014	<0.010	0.027	0.034	0.021	0.036	0.036	0.015	0.064
3-methylisoquinoline	0.034	0.030	0.063	0.041	n.a	0.051	<0.004	<0.004	<0.004	0.017	0.020	<0.004	0.012	0.014	<0.004
2-methylquinoline	0.016	0.013	0.235	0.118	n.a	0.321	0.086	<0.006	0.092	0.055	0.073	0.101	0.057	0.052	0.117
4-methylquinoline	0.012	0.007	0.404	0.218	n.a	0.499	0.177	<0.004	0.096	0.108	0.155	0.394	0.128	0.109	0.346
6-methylquinoline	0.010	0.007	0.041	0.022	n.a	0.032	<0.004	<0.004	<0.004	0.016	<0.004	<0.004	0.016	0.014	0.097
2(1H)-quinolinone	0.019	0.021	0.069	0.038	n.a	1.60	0.029	<0.010	1.05	0.019	0.191	<0.010	0.012	0.019	1.62
1(2H)-isoquinolinone	0.252	0.262	0.401	0.147	n.a	2.51	<0.034	<0.034	10.3	<0.034	13.6	5.10	<0.034	0.344	169
4(1H)-quinolinone	0.013	0.010	<0.008	<0.008	n.a	0.100	0.010	<0.008	0.114	<0.008	0.025	<0.008	<0.008	0.016	<0.008
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	n.a	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	n.a	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	n.a	<0.004	<0.004	<0.004	<0.004	<0.004	0.003	<0.004	<0.004	<0.004	0.392
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	n.a	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3,4-dihydro-2(1H)-quinolinone	<0.008	<0.008	<0.008	<0.008	n.a	0.316	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	0.539
3,4-dihydro-1(2H)-isoquinolinone	0.064	0.075	0.139	0.054	n.a	0.089	0.011	<0.006	<0.006	0.015	0.030	0.200	0.012	<0.006	<0.006
2-methyl-6-quinolinol	<0.003	<0.003	<0.003	<0.003	n.a	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	0.012	0.005	0.071	<0.006	n.a	0.318	0.036	<0.006	18.9	0.041	1.50	3.21	<0.006	0.564	18.5
6-methoxyquinoline	0.004	<0.003	0.025	0.015	n.a	0.018	0.010	<0.003	0.031	0.010	0.010	0.029	0.009	0.013	<0.003
1-methyl-2-quinolinone	0.081	0.060	0.101	0.113	n.a	0.101	0.010	0.008	<0.005	0.053	0.296	<0.005	0.036	0.079	9.11
2-methyl-4(1H)-quinolinone	0.579	0.610	0.839	0.131	n.a	0.409	<0.006	<0.006	1.38	<0.006	0.217	1.54	<0.006	0.080	3.38
2,4-dimethylquinoline	0.010	0.007	0.403	0.200	n.a	0.483	0.198	<0.006	0.269	0.177	0.205	0.497	0.189	0.180	0.497
2,6-dimethylquinoline	0.006	<0.006	0.036	0.028	n.a	0.035	0.014	<0.006	0.041	0.019	0.016	<0.006	0.016	0.019	<0.006
1,2,3,4-tetrahydroquinoline	0.034	0.028	<0.013	<0.013	n.a	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013
7-hydroxy-4-methyl-2(1H)-quinolinone	0.193	0.202	0.233	<0.005	n.a	<0.005	<0.005	0.013	<0.005	0.125	<0.005	<0.005	<0.005	<0.005	<0.005
4-hydroxy-1-methyl-2(1H)-quinolinone	0.306	0.302	0.303	0.556	n.a	0.326	<0.006	0.022	<0.006	<0.006	0.160	0.713	0.010	0.024	0.761

n.a. = not analysed

Castrop-Rauxel: HPLC data downstream the testfield

Table 28: HPLC-DAD and MS-MS data from the Castrop-Rauxel field site; wells ~100 m distance from the third line of the testfield; sampling March 2005.

compound	T19t	T19b	T18t	T18b
carbazole	0.025	0.125	0.04	0.145
6(5H)-phenanthridinone	<0.04	<0.04	<0.04	<0.04
acridine	<0.24	<0.24	<0.24	<0.24
9(10H)-acridinone	<0.24	<0.24	<0.24	<0.24
benzothiophene	0.90	2.00	2.80	2.10
3-methylbenzothiophene	<0.08	<0.08	<0.08	<0.08
dibenzothiophene	<0.08	<0.08	<0.08	<0.08
benzofuran	<0.04	<0.04	0.40	<0.04
2-methylbenzofuran	<0.04	<0.04	<0.04	<0.04
methylbenzofuran isomer 1	<0.04	<0.04	<0.04	<0.04
2,3-dimethylbenzofuran	<0.04	<0.04	<0.04	<0.04
dibenzofuran	0.150	0.35	<0.04	<0.04
naphthalene	4.40	10.25	11.90	10.35
1-methylnaphthalene	0.60	1.30	1.30	1.40
2-methylnaphthalene	0.08	0.10	0.15	0.10
1,3-dimethylnaphthalene	<0.04	<0.04	<0.04	<0.04
2-naphthol	<0.04	<0.04	<0.04	<0.04
1-naphthol	<0.04	<0.04	<0.04	<0.04
2-naphthoic acid	<0.12	<0.12	<0.12	<0.12
fluorene	<0.06	<0.06	<0.06	<0.06
acenaphthene	0.15	0.35	0.30	0.55
acenaphthenol	<0.02	<0.02	<0.02	<0.02
indene	1.25	2.25	4.20	3.20
indan	1.20	1.80	2.60	2.25
1-indanone	<0.04	0.90	0.34	0.70
quinoline	<0.0006	0.015	0.015	0.020
isoquinoline	0.014	0.051	0.057	0.075
1-methylisoquinoline	<0.001	0.010	<0.001	0.021
3-methylisoquinoline	<0.004	0.010	0.011	0.014
2-methylquinoline	<0.0006	0.019	0.026	<0.0006
4-methylquinoline	0.003	0.009	0.011	0.015
6-methylquinoline	0.002	<0.0004	<0.0004	<0.0004
2(1H)-quinolinone	0.016	0.038	0.020	0.062
1(2H)-isoquinolinone	0.030	0.037	0.052	0.143
4(1H)-quinolinone	0.009	0.016	0.010	0.024
5(1H)-quinolinone	<0.0007	<0.0007	<0.0007	<0.0007
6-quinolinol	<0.0004	<0.0004	<0.0004	<0.0004
7-isoquinolinol	<0.0004	<0.0004	<0.0004	<0.0004
3(2H)-isoquinolinone	<0.0004	<0.0004	<0.0004	<0.0004
3,4-dihydro-2(1H)-quinolinone	0.008	<0.0008	0.013	<0.0008
3,4-dihydro-1(2H)-isoquinolinone	<0.0006	<0.0006	0.006	<0.0006
2-methyl-6-quinolinol	<0.0003	<0.0003	<0.0003	<0.0003
4-methyl-2(1H)-quinolinone	0.007	0.035	0.021	0.046
6-methoxyquinoline	<0.0003	<0.0003	<0.0003	<0.0003
1-methyl-2-quinolinone	<0.0005	<0.0005	<0.0005	<0.0005
2-methyl-4(1H)-quinolinone	<0.0006	<0.0006	<0.0006	<0.0006
2,4-dimethylquinoline	<0.0006	0.010	0.011	0.014
2,6-dimethylquinoline	<0.0006	<0.0006	<0.0006	<0.0006
1,2,3,4-tetrahydroquinoline	<0.0013	<0.0013	<0.0013	<0.0013
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.0005	<0.0005	<0.0005	<0.0005
4-hydroxy-1-methyl-2(1H)-quinolinone	0.018	0.026	0.021	0.041

t= top; b = bottom

Castrop-Rauxel: Distribution of compounds within the testfield

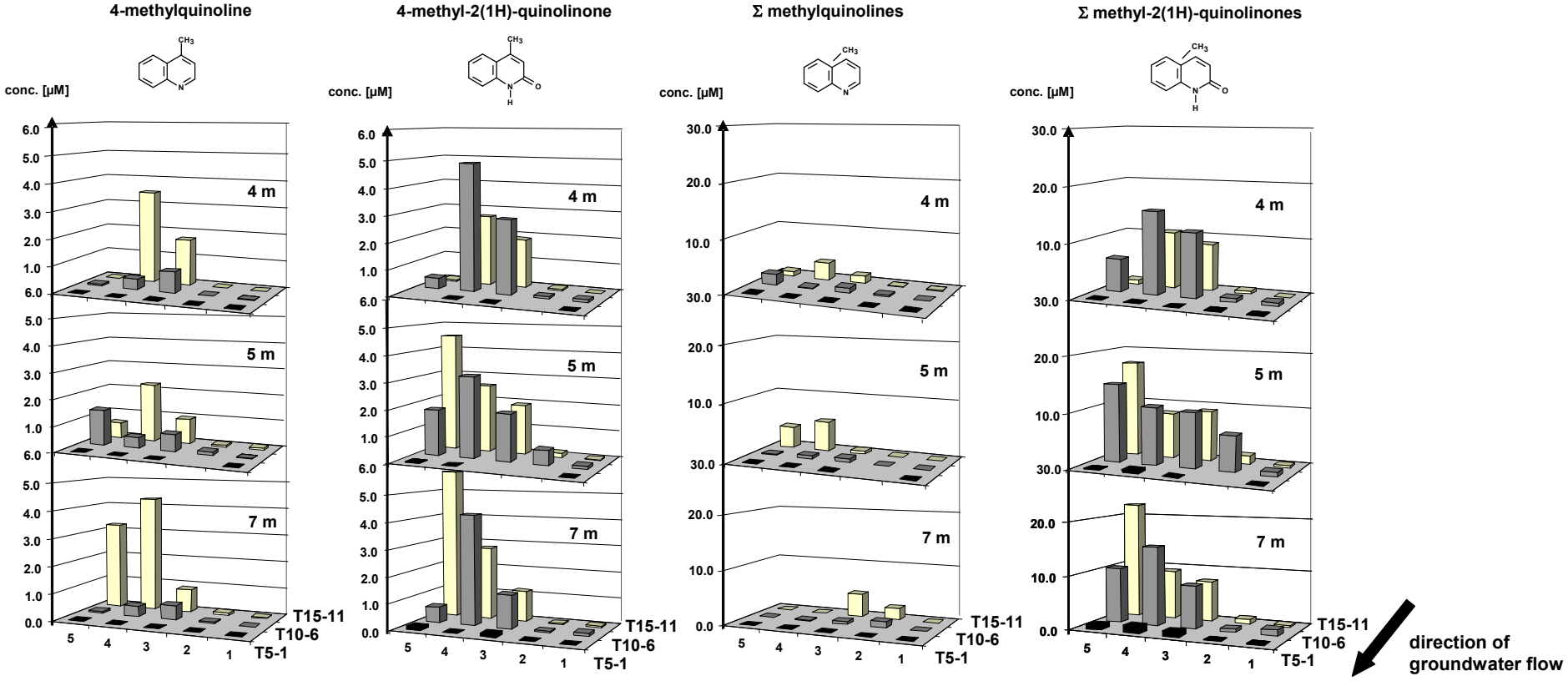


Figure 48: Distribution of 4-methylquinoline and 4-methyl-2(1H)-quinolinone as well as sum of methylquinolines and methyl-2(1H)-quinolinones within the testfield of Castrop-Rauxel. Methylquinolines: 1 nM = 143 ng/L; methyl-2(1H)-quinolinones 1 nM = 159 ng/L.

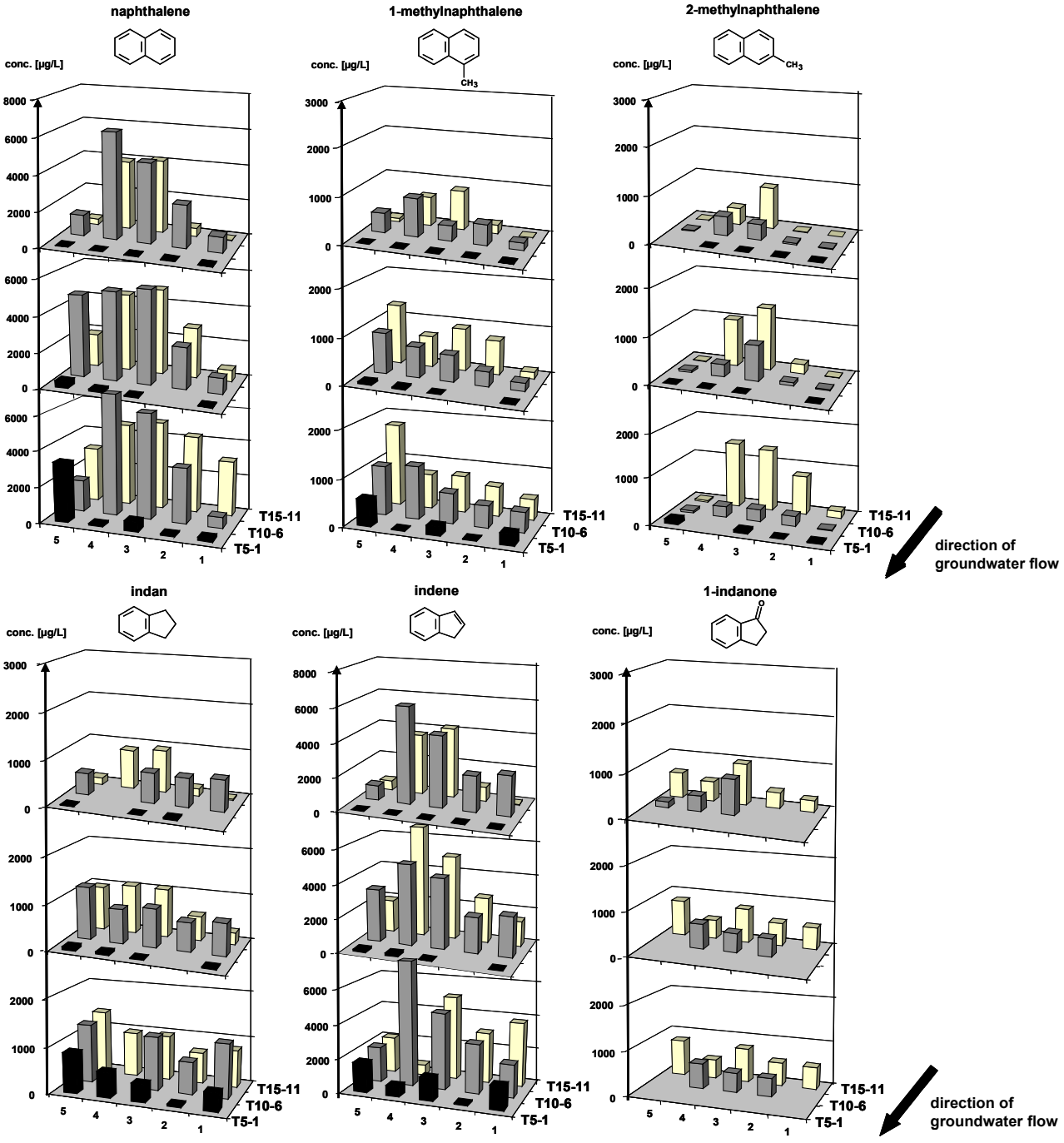


Figure 49: Distribution of naphthalene and 1- and 2-methylnaphthalene as well as homocycles indan, indene and 1-indanone within the testfield of Castrop-Rauxel.

Castrop-Rauxel: ^{1}H NMR and ^{13}C NMR data of the groundwater sample

Table 29: ^{1}H NMR, ^{13}C NMR and MS data of 2(1H)-quinolinone, 1(2H)-isoquinolinone and methyl-2(1H)-quinolinones as well as 3,4-dihydro-2(1H)-quinolinone and methyl-3,4-dihydro-2(1H)-quinolinones identified in an enriched groundwater sample of the tar oil contaminated site in Castrop-Rauxel (^{1}H and ^{13}C NMR data were generated off-line from the isolated compounds).

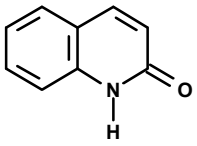
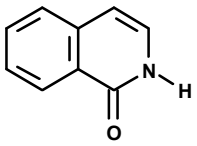
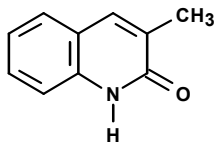
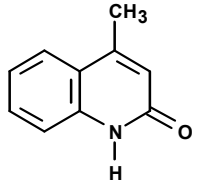
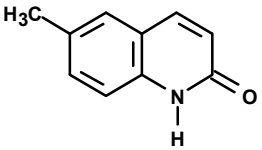
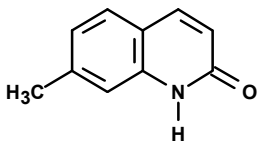
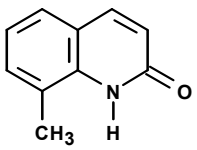
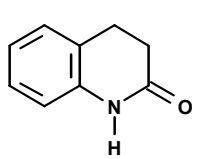
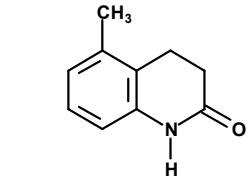
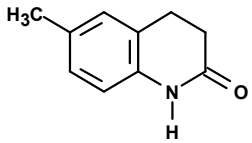
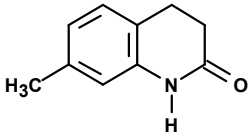
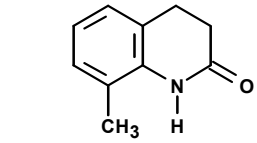
	**2(1H)-Quinolinone*** ^{1}H NMR, δ (ppm): NH: 11.66 (s), H-4: 7.76 (d, $^{3}J_{4,3}$ = 9.6 Hz), H-5: 7.54 (d, $^{3}J_{5,6}$ = 7.8 Hz), H-7: 7.41 (dd, $^{3}J_{7,6}$ = 7.2 Hz, $^{3}J_{7,8}$ = 8.4 Hz), H-8: 7.30(d, $^{3}J_{8,7}$ = 8.4 Hz), H-6: 7.11 (dd, $^{3}J_{6,7}$ = 7.2 Hz, $^{3}J_{6,5}$ = 7.8 Hz) $ESI_{pos.}$-MS, m/z: 146 $[M+H]^{+}$
	**1(2H)-Isoquinolinone*** ^{1}H NMR, δ (ppm): NH: 11.11 (s), H-8: 8.20 (d, $^{3}J_{8,7}$ = 7.8 Hz), H-6: 7.61 (t, $^{3}J_{6,5}$ = 7.8 Hz, $^{3}J_{6,7}$ = 7.2 Hz), H-5: 7.54 (d, $^{3}J_{5,6}$ = 7.8 Hz), H-7: 7.41(t, $^{3}J_{7,8}$ = 7.8 Hz, $^{3}J_{7,6}$ = 7.2 Hz), H-3: 7.05 (d, $^{3}J_{3,4}$ = 7.2 Hz), H-4: 6.45 (d, $^{3}J_{4,3}$ = 7.2 Hz) $ESI_{pos.}$-MS, m/z:146 $[M+H]^{+}$
	**3-Methyl-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 11.64 (s), H-4: 7.62 (s), H-5: 7.46 (dd, $^{3}J_{5,6}$ = 8.0 Hz, $^{4}J_{5,7}$ = 1.0 Hz), H-7: 7.33 (ddd, $^{3}J_{7,8}$ = 8.4 Hz, $^{3}J_{7,6}$ = 7.0 Hz, $^{4}J_{7,5}$ = 1.0 Hz), H-8: 7.27 (dd, $^{3}J_{8,7}$ = 8.4 Hz, $^{4}J_{8,6}$ = 1.0 Hz), H-6: 6.34 (ddd, $^{3}J_{6,5}$ = 8.0 Hz, $^{3}J_{6,7}$ = 7.0 Hz, $^{4}J_{6,8}$ = 1.0 Hz), CH_3: 2.11 (s) $ESI_{pos.}$-MS, m/z: 160 $[M+H]^{+}$ ^{13}C NMR, δ (ppm) from HMQC and HMBC spectra: C-2: 163,4, C-9: 138.5, C-4: 136.8, C-3: 130.5, C-7: 129.3, C-5: 127.4, C-6: 122.0, C-10: 120.2, C-8: 115.5, CH_3: 17.1
	**4-Methyl-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 11.50 (s), H-5: 7.62 (dd, $^{3}J_{5,6}$ = 8.0 Hz, $^{4}J_{5,7}$ = 1.0 Hz), H-7: 7.41 (ddd, $^{3}J_{7,8}$ = 8.4 Hz, $^{3}J_{7,6}$ = 7.0 Hz, $^{4}J_{7,5}$ = 1.0 Hz), H-8: 7.29 (dd, $^{3}J_{8,7}$ = 8.4 Hz, $^{4}J_{8,6}$ = 1.0 Hz,), H-6: 7.14 (ddd, $^{3}J_{6,5}$ = 8.0 Hz, $^{3}J_{6,7}$ = 7.0 Hz, $^{4}J_{6,4}$ = 1.0 Hz), H-3: 6.34 (s), CH_3: 2.42 (s) $ESI_{pos.}$-MS, m/z: 160 $[M+H]^{+}$
	**6-Methyl-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 11.57 (s), H-4: 7.69 (d, $^{3}J_{4,3}$ = 9.6 Hz), H-5: 7.32 (s, broad), H-7: 7.23 (dd, $^{3}J_{7,8}$ = 8.4 Hz, $^{4}J_{7,5}$ = 1.4 Hz), H-8: 7.19 (d, $^{3}J_{8,7}$ = 8.4 Hz), H-3: 6.42 (d, $^{3}J_{3,4}$ = 9.6 Hz), CH_3: 2.33 (s) $ESI_{pos.}$-MS, m/z: 160 $[M+H]^{+}$ ^{13}C NMR, δ (ppm) from HMQC and HMBC spectra: C-2: 163.0, C-4: 140.3, C-9: 137.5, C-7: 132.1, C-6: 131.6, C-5: 128.0, C-3: 122.2, C-10: 119.8, C-8: 115.9, CH_3: 21.0
	**7-Methyl-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 11.56 (s), H-4: 7.71 (d, $^{3}J_{4,3}$ = 9.0 Hz), H-5: 7.42 (d, $^{3}J_{5,6}$ = 8.0 Hz), H-8: 7.08 (s, broad), H-6: 6.93 (dd, $^{3}J_{6,5}$ = 8.0 Hz, $^{4}J_{6,8}$ = 1.0 Hz), H-3: 6.37 (d, $^{3}J_{3,4}$ = 9.0 Hz), CH_3: 2.37 (s) $ESI_{pos.}$-MS, m/z: 160 $[M+H]^{+}$
	**8-Methyl-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 10.72 (s), H-4: 7.76 (d, $^{3}J_{4,3}$ = 9.0 Hz), H-5: 7.39 (d, $^{3}J_{5,6}$ = 7.4 Hz), H-7: 7.27 (d, $^{3}J_{7,6}$ = 7.4 Hz), H-6: 7.03 (t, $^{3}J_{6,5}$ = $^{3}J_{6,7}$ = 7.4 Hz), H-3: 6.46 (d, $^{3}J_{3,4}$ = 9.0 Hz), CH_3: 2.43 (s) $ESI_{pos.}$-MS, m/z: 160 $[M+H]^{+}$ ^{13}C NMR, δ (ppm) from HMQC and HMBC spectra: C-2: 163,4, C-4: 141.2, C-9: 137.9, C-7: 132.3, C-5: 126.3, C-8: 124.1, C-3, C-6: 122.1, C-10: 119.9, CH_3: 17.9
	**3,4-Dihydro-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 9.94 (s), H-5: 7.08 (d, $^{3}J_{5,6}$ = 7.8 Hz), H-7: 7.06 (dd, $^{3}J_{7,6}$ = 7.2 Hz, $^{3}J_{7,8}$ = 7.8 Hz), H-6: 6.85 (t, $^{3}J_{6,5}$ = 7.8 Hz, $^{3}J_{6,7}$ = 7.2 Hz), H-8: 6,83 (d, $^{3}J_{8,7}$ = 7.8 Hz), H-4a,b: 2.84-2.88 (pt), H-3a,b: 2.41-2.46 (pt) $ESI_{pos.}$-MS, m/z: 148 $[M+H]^{+}$ ^{13}C NMR, δ (ppm) from HMQC and HMBC spectra: C-2: 171.0, C-9: 138.9, C-5: 128.2, C-7: 127.7, C-10: 124.0, C-6: 122.6, C-8: 115.8, C-3: 31.3, C-4: 25.8

Table 29, continued (part 2).

	**5-Methyl-3,4-dihydro-2(1H)-quinolinone** ^{1}H NMR, δ (ppm): NH: 9.87 (s), H-7: 6.95 (t, $^3J_{7,6}$ = 7.8 Hz, $^3J_{7,8}$ = 7,8 Hz), H-6: 6.73 (d, $^3J_{6,7}$ = 7.8 Hz), H-8: 7.68 (d, $^3J_{8,7}$ = 7.8 Hz), H-4a,b: 2.81 (t), H-3a,b: 2.44 (t), CH_3: 2.21 (s) $ESI_{pos.}$-MS, m/z: 162 $[M+H]^+$
	**6-Methyl-3,4-dihydro-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 9,84 (s), H-5: 6.90 (s, broad), H-7: 6.86 (d, $^3J_{7,8}$ = 7.8 Hz), H-8: 6.71 (d, $^3J_{8,7}$ = 7.8 Hz), H-4a,b: 2.80-2.83 (pt), H-3a,b: 2.39-2.43 (pt), CH_3: 2.21 (s) $ESI_{pos.}$-MS, m/z: 162 $[M+H]^+$ ^{13}C NMR, δ (ppm) from HMQC and HMBC spectra: C-2: 170.8, C-9: 136.5, C-6: 131.7, C-5: 128.8, C-7: 128.1, C-10: 123.9, C-8: 115.7, C-3: 31.4, C-4: 25.7, CH_3: 21.2
	**7-Methyl-3,4-dihydro-2(1H)-quinolinone*** ^{1}H NMR, δ (ppm): NH: 9,87 (s), H-5: 6.95 (d, $^3J_{5,6}$ = 7.8 Hz), H-6: 6.67 (d, $^3J_{6,5}$ = 7.8 Hz), H-8: 6.64 (s, broad), H-4a,b: 2.79-2.82 (pt), H-3a,b: 2.40-2.43 (pt), CH_3: 2.21 (s) $ESI_{pos.}$-MS, m/z: 162 $[M+H]^+$ ^{13}C NMR, δ (ppm) from HMQC and HMBC spectra: C-2: 170.8, C-9: 138.9, C-7: 137.0, C-5: 128.1, C-6: 123.3, C-10: 121.0, C-8: 116.4, C-3: 31.4, C-4: 25.6, CH_3: 21.4
	**8-Methyl-3,4-dihydro-2(1H)-quinolinone** ^{1}H NMR, δ (ppm): NH: 9.18 (s), H-6: 6.79 (t, $^3J_{6,5}$ = 7.8 Hz, $^3J_{6,7}$ = 7,8 Hz), H-5,H-7: 6.92-6.95, CH_3: 2.20 (s) $ESI_{pos.}$-MS, m/z: 162 $[M+H]^+$

*metabolites, which were also formed in microcosms.

7.4.2 Düsseldorf-Flingern: Field data

Düsseldorf-Flingern: HPLC-DAD data

Table 30: HPLC-DAD data from the Düsseldorf-Flingern field site; sampling October 2005. Concentration [µg/L].

compound	19200				19201					19202				
	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m
carbazole	41	<0.4	<0.4	<0.4	<0.4	<0.4	18	34	35	31	71	19	13	16
6(5H)-phenanthridinone	12	5.1	5.3	2.2	91	25	8.0	7.1	8.5	19	10	3.2	3.2	2.5
acridine	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
9(10H)-acridinone	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	7.5	6.6	12	13	7.6	<2.4	<2.4
benzothiophene	62	8.2	21	7.2	550	127	18	29	23	72	7.1	8.9	11	38
3-methylbenzothiophene	135	12	30	8.5	173	173	70	49	49	137	71	<0.8	31	25
methylbenzothiophene isomer 1	55	2.0	8.0	<0.8	82	82	13	9.1	11	48	13	<0.8	4.2	5.5
methylbenzothiophene isomer 2	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
methylbenzothiophene isomer 3	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
dimethylbenzothiophene isomer 1	19	8.2	14	5.5	<0.8	<0.8	25	<0.8	<0.8	32	19	8.4	10	6.9
dimethylbenzothiophene isomer 2	16	7.2	11	5.0	<0.8	<0.8	28	<0.8	<0.8	28	21	7.5	12	4.6
dibenzothiophene	<0.8	11	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	26	<0.8	<0.8	<0.8	22	17
benzofuran	7.4	<0.4	<0.4	<0.4	546	90	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-methylbenzofuran	15	<0.4	<0.4	<0.4	<0.4	41	<0.4	<0.4	<0.4	12	<0.4	<0.4	<0.4	<0.4
methylbenzofuran isomer 1	<0.4	9.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
methylbenzofuran isomer 2	7.4	2.2	<0.4	<0.4	<0.4	<0.4	3.7	0.7	1.8	<0.4	<0.4	1.0	<0.4	<0.4
methylbenzofuran isomer 3	9.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	1.3	<0.4
methylbenzofuran isomer 4	40	14	17	7.2	<0.4	41	17	17	14	17	<0.4	4.7	6.9	9.0
2,3-dimethylbenzofuran	<0.4	4.4	<0.4	2.6	<0.4	<0.4	21	<0.4	12	<0.4	<0.4	6.7	9.6	<0.4
dimethylbenzofuran isomer	39	<0.4	7.9	<0.4	<0.4	40.3	22	12	13	46	<0.4	<0.4	12	<0.4
dibenzofuran	404	13	29	9.3	536	881	61	253	18	965	876	15	22	95
methyldibenzofuran isomer 1	<0.4	6.0	8.8	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	13	<0.4	10	13	9.9
methyldibenzofuran isomer 2	<0.4	21	33	39	<0.4	<0.4	46	72	56	56	38	48	56	49
methyldibenzofuran isomer 3	<0.4	26	37	37	<0.4	<0.4	55	71	51	58	<0.4	49	58	44
naphthalene	286	10	11	4.5	3673	429	33	37	2.2	119	32	14	13	16
1-methylnaphthalene	445	5.9	14	4.9	1303	1328	24	<0.4	7.9	345	52	7.8	6.3	11
2-methylnaphthalene	<0.4	<0.4	<0.4	<0.4	649	<0.4	<0.4	<0.4	4.5	<0.4	<0.4	<0.4	<0.4	<0.4
1,3-dimethylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	<0.4	<0.4	<0.4	<0.4	17	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthoic acid	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
fluorene	92	86	56	118	195	293	233	477	354	162	606	322	287	277
acenaphthene	578	448	546	341	768	1082	974	1172	625	1336	1250	344	608	344
acenaphthenol	<0.4	1.0	2.3	2.2	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	1.0	1.6	1.4
indene	129	3.7	6.5	3.3	952	366	6.5	11	5.2	25	<0.4	<0.4	<0.4	3.8
indan	<1.2	<1.2	<1.2	<1.2	<1.2	215	<1.2	<1.2	<1.2	134	<1.2	<1.2	<1.2	<1.2
1-indanone	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

Table 30, continued (part 2). Concentration [µg/L].

compound	19203					19214					19185		
	6.5 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	8 m	9 m	10 m
carbazole	56	71	24	16	<0.4	139	73	6.9	9.9	<0.4	<0.4	<0.4	10
6(5H)-phenanthridinone	15	12	5.0	<0.4	<0.4	14	4.3	<0.4	<0.4	<0.4	46	8.5	4.4
acridine	<0.4	<0.4	<0.4	<0.4	3.0	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
9(10H)-acridinone	6.8	7.5	11	7.3	7.4	6.7	<2.4	7.8	9.8	6.4	<2.4	<2.4	<2.4
benzothiophene	147	30	17	9.1	7.3	834	359	7.8	14	7.9	104	13	24
3-methylbenzothiophene	226	100	34	49	27	528	160	3.6	<0.8	<0.8	49	36	132
methylbenzothiophene isomer 1	72	25	6.3	10	<0.8	269	63	<0.8	<0.8	<0.8	9.5	6.5	43
methylbenzothiophene isomer 2	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	8.4	<0.8	<0.8	<0.8	<0.8	<0.8
methylbenzothiophene isomer 3	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	5.8	<0.8	<0.8	<0.8	<0.8	<0.8
dimethylbenzothiophene	26	20	16	15	9.9	<0.8	<0.8	<0.8	<0.8	<0.8	26	12	11
dimethylbenzothiophene	20	17	17	13	9.8	<0.8	29	4.2	6.4	4.7	27	12	<0.8
dibenzothiophene	<0.8	<0.8	15	37	23	<0.8	<0.8	13	13	12	<0.8	<0.8	28
benzofuran	<0.4	<0.4	<0.4	<0.4	<0.4	16	9.5	<0.4	<0.4	<0.4	16	<0.4	<0.4
2-methylbenzofuran	20	<0.4	<0.4	<0.4	<0.4	175	62	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
methylbenzofuran isomer 1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
methylbenzofuran isomer 2	3.6	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	12	<0.4	1.5
methylbenzofuran isomer 3	12	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	2.2	<0.4	2.2	<0.4	2.2
methylbenzofuran isomer 4	17	<0.4	3.3	3.3	4.4	<0.4	37	<0.4	4.2	<0.4	36	9.4	17
2,3-dimethylbenzofuran	<0.4	7.6	14	14	7.6	<0.4	<0.4	<0.4	9.0	<0.4	<0.4	12	<0.4
dimethylbenzofuran isomer	57	<0.4	<0.4	<0.4	7.6	90	72	<0.4	<0.4	<0.4	45	12	20
dibenzofuran	788	900	36	30	25	337	360	6.8	7.1	4.5	32	9.3	57
methyldibenzofuran isomer 1	<0.4	<0.4	17	<0.4	12	<0.4	<0.4	7.6	7.1	8.3	<0.4	12	13
methyldibenzofuran isomer 2	37	58	80	83	58	<0.4	41	31	32	38	<0.4	26	66
methyldibenzofuran isomer 3	<0.4	<0.4	70	78	56	<0.4	<0.4	33	28	39	<0.4	50	57
naphthalene	282	19	15	12	5.2	4352	2943	33	23	18	414	16	15
1-methylnaphthalene	1347	194	15	26	6.1	1120	662	6.9	5.8	4.4	146	7.5	7.4
2-methylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	211	63	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1,3-dimethylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthol	6.2	<0.4	<0.4	<0.4	<0.4	10	4.5	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	11	8.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthoic acid	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
fluorene	215	442	567	487	310	237	424	161	136	91	7.9	61	319
acenaphthene	1063	136	900	823	558	515	638	221	318	256	1050	638	642
acenaphthenol	1.1	1.7	1.8	1.1	1.4	<0.4	0.7	0.8	<0.4	<0.4	<0.4	<0.4	<0.4
indene	20	7.5	3.7	3.6	2.7	295	54	2.5	<0.4	<0.4	128	11	4.5
indan	189	50	<1.2	<1.2	<1.2	<1.2	297	<1.2	<1.2	<1.2	44	170	<1.2
1-indanone	<0.4	<0.4	<0.4	<0.4	<0.4	7.1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

Table 30, continued (part 3). Concentration [µg/L].

compound	19064			19206			
	10 m	12 m	13 m	7 m	8 m	9 m	10 m
carbazole	<0.4	<0.4	<0.4	<0.4	<0.4	8.1	<0.4
6(5H)-phenanthridinone	<0.4	<0.4	<0.4	<0.4	5.9	4.0	<0.4
acridine	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
9(10H)-acridinone	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
benzothiophene	<0.8	<0.8	<0.8	32	35	23	<0.8
3-methylbenzothiophene	<0.4	<0.4	<0.4	32	55	45	<0.8
methylbenzothiophene isomer 1	<0.4	<0.4	<0.4	7.8	18	9.4	<0.8
methylbenzothiophene isomer 2	<0.4	<0.4	<0.4	<0.8	<0.8	<0.8	<0.8
methylbenzothiophene isomer 3	<0.4	<0.4	<0.4	<0.8	<0.8	<0.8	<0.8
dimethylbenzothiophene	2.6	2.0	2.5	<0.8	<0.8	<0.8	<0.8
dimethylbenzothiophene	<0.8	<0.8	<0.8	<0.8	12	<0.8	<0.8
dibenzothiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
benzofuran	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-methylbenzofuran	<0.4	<0.4	<0.4	9.3	<0.4	<0.4	<0.4
methylbenzofuran isomer 1	<0.4	<0.4	0.6	<0.4	<0.4	<0.4	<0.4
methylbenzofuran isomer 2	<0.4	<0.4	<0.4	<0.4	7.6	2.6	<0.4
methylbenzofuran isomer 3	<0.4	<0.4	<0.4	<0.4	2.4	<0.4	<0.4
methylbenzofuran isomer 4	2.6	0.9	<0.4	<0.4	26	26	3.1
2,3-dimethylbenzofuran	2.2	1.1	0.8	<0.4	<0.4	15.	2.8
dimethylbenzofuran isomer	2.2	1.0	0.6	5.7	<0.4	<0.4	<0.4
dibenzofuran	2.2	2.2	<0.4	<0.4	<0.4	35	3.9
methyldibenzofuran isomer 1	3.6	3.0	3.2	<0.4	<0.4	12	6.0
methyldibenzofuran isomer 2	11	7.1	4.8	<0.4	<0.4	49	23
methyldibenzofuran isomer 3	13	9.3	<0.4	<0.4	<0.4	53	27
naphthalene	2.2	2.1	<0.2	3.3	4.7	7.9	<0.2
1-methylnaphthalene	2.1	2.1	2.2	23	85	12	2.5
2-methylnaphthalene	3.6	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1,3-dimethylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthoic acid	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
fluorene	<0.6	<0.6	<0.6	2.7	87	61	7.4
acenaphthene	123	80	37	13	746	638	247
acenaphthenol	<0.4	<0.4	<0.4	<0.4	3.0	1.5	1.0
indene	<0.4	<0.4	<0.4	4.5	5.1	7.9	<0.4
indan	<1.2	<1.2	<1.2	38	53	<1.2	<1.2
1-indanone	<0.4	<0.4	<0.4	<0.4	5.4	<0.4	<0.4

Table 30, continued (part 4). Concentration [µg/L].

compound	19213				19186					19205				
	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m
carbazole	19	16	21	20	5.2	4.7	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
6(5H)-phenanthridinone	<0.4	20	5.7	<0.4	5.5	2.5	1.3	<0.4	<0.4	9.9	18	16	<0.4	<0.4
acridine	<0.4	<0.4	<0.4	<0.4	3.9	2.1	<0.4	2.5	0.0	<0.4	<0.4	<0.4	<0.4	<0.4
9(10H)-acridinone	6.9	6.3	5.8	7.4	<2.4	<2.4	<2.4	2.4	4.5	9.7	16	3.6	<2.4	<2.4
benzothiophene	74	<0.8	23	19	4.0	<0.8	<0.8	<0.8	<0.8	6.6	<0.8	<0.8	<0.8	3.3
3-methylbenzothiophene	103	37	15	28	<0.8	<0.8	<0.8	<0.8	7.8	15	44	29	32	5.4
methylbenzothiophene isomer 1	32	<0.8	<0.8	4.1	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	8.1	4.1	<0.8	<0.8
methylbenzothiophene isomer 2	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
methylbenzothiophene isomer 3	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
dimethylbenzothiophene	24	13	<0.8	14	<0.8	<0.8	<0.8	<0.8	7.4	<0.8	<0.8	<0.8	<0.8	<0.8
dimethylbenzothiophene	16	9.7	<0.8	12	<0.8	<0.8	<0.8	<0.8	3.4	3.4	10	9.0	42	<0.8
dibenzothiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
benzofuran	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-methylbenzofuran	<0.4	<0.4	<0.4	<0.4	1.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
methylbenzofuran isomer 1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
methylbenzofuran isomer 2	4.6	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	1.1	<0.4	2.9	<0.4	<0.4	<0.4
methylbenzofuran isomer 3	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	2.5	<0.4	<0.4	<0.4
methylbenzofuran isomer 4	16	<0.4	<0.4	5.1	<0.4	1.0	<0.4	<0.4	2.3	4.1	11	8.6	<0.4	2.0
2,3-dimethylbenzofuran	<0.4	<0.4	<0.4	8.8	<0.4	1.0	<0.4	<0.4	2.3	5.2	<0.4	6.6	12	2.7
dimethylbenzofuran isomer	35	<0.4	<0.4	<0.4	4.3	0.9	<0.4	<0.4	<0.4	5.4	15	11	18	3.2
dibenzofuran	21	5.7	6.2	7.7	83	6.0	3.0	3.0	4.8	4.5	<0.4	2.5	2.1	3.2
methyldibenzofuran isomer 1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	2.2	<0.4	<0.4	<0.4	<0.4	<0.4	29	4.5
methyldibenzofuran isomer 2	17	30	46	69	<0.4	5.0	4.9	3.5	21	2.6	<0.4	<0.4	43	17
methyldibenzofuran isomer 3	<0.4	<0.4	48	70	<0.4	4.3	<0.4	3.2	22	<0.4	<0.4	<0.4	68	18
naphthalene	9.7	<0.2	<0.2	<0.2	<0.2	<0.2	2.5	3.0	<0.2	5.3	<0.2	<0.2	<0.2	<0.2
1-methylnaphthalene	79	5.7	4.6	3.8	8.7	2.6	3.1	2.9	3.2	5.3	<0.4	4.3	2.0	2.3
2-methylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	1.8	<0.4	<0.4	<0.4	<0.4
1,3-dimethylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthoic acid	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
fluorene	65	510	373	389	65	36	2.6	2.6	33	<0.6	7.1	<0.6	65	<0.6
acenaphthene	486	778	810	703	79	25	9.2	7.9	265	185	473	422	640	171
acenaphthenol	<0.4	2.4	1.8	2.3	<0.4	<0.4	<0.4	<0.4	1.1	1.0	1.7	2.7	21.7	0.9
indene	10	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	2.5
indan	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
1-indanone	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

Table 30, continued (part 5). Concentration [µg/L].

compound	19209				19210					19071*
	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	
carbazole	<0.4	<0.4	<0.4	18	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
6(5H)-phenanthridinone	<0.4	<0.4	9.6	5.8	<0.4	41	25	<0.4	<0.4	<0.04
acridine	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
9(10H)-acridinone	<2.4	<2.4	<2.4	6.5	<2.4	<2.4	<2.4	<2.4	<2.4	<0.24
benzothiophene	32	447	65	16	<0.8	17	<0.8	<0.8	<0.8	3.0
3-methylbenzothiophene	<0.4	<0.4	20	<0.4	4.7	52	<0.4	<0.4	<0.4	4.9
methylbenzothiophene isomer 1	7.7	<0.4	12	<0.4	1.7	13	<0.4	<0.4	<0.4	<0.04
methylbenzothiophene isomer 2	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
methylbenzothiophene isomer 3	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
dimethylbenzothiophene	<0.8	<0.8	25	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	65
dimethylbenzothiophene	<0.8	<0.8	15	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.08
dibenzothiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.08
benzofuran	<0.4	30	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
2-methylbenzofuran	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
methylbenzofuran isomer 1	<0.4	<0.4	<0.4	<0.4	<0.4	8.9	<0.4	<0.4	<0.4	<0.04
methylbenzofuran isomer 2	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
methylbenzofuran isomer 3	<0.4	153	13	<0.4	<0.4	<0.4	3.3	2.8	1.4	12
methylbenzofuran isomer 4	10	<0.4	30	15	<0.4	<0.4	<0.4	<0.4	<0.4	26
2,3-dimethylbenzofuran	<0.4	77	<0.4	17	18	151	24	22	9.6	<0.04
dimethylbenzofuran isomer	<0.4	<0.4	<0.4	13	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
dibenzofuran	<0.4	<0.4	15	13	3.1	3.5	3.1	2.7	2.6	0.6
methyldibenzofuran isomer 1	<0.4	<0.4	<0.4	13	<0.4	<0.4	5.7	4.2	3.4	14
methyldibenzofuran isomer 2	<0.4	<0.4	<0.4	59	<0.4	<0.4	22	22	13	39
methyldibenzofuran isomer 3	<0.4	<0.4	<0.4	61	<0.4	<0.4	36	24	15	65
naphthalene	13	821	134	<0.2	3.0	<0.2	<0.2	<0.2	<0.2	2.2
1-methylnaphthalene	17	430	100	3.3	2.7	<0.4	3.2	2.7	2.6	24
2-methylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
1,3-dimethylnaphthalene	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
2-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
1-naphthol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04
2-naphthoic acid	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<0.12
fluorene	<0.6	<0.6	27	275	2.6	<0.6	<0.6	<0.6	<0.6	3.7
acenaphthene	<1.2	<1.2	772	914	5.6	250	318	203	122	78
acenaphthenol	<0.4	<0.4	<0.4	<0.4	<0.4	2.2	1.5	<0.4	<0.4	<0.04
indene	29	207	13	<0.4	<0.4	6.2	<0.4	<0.4	<0.4	<0.04
indan	103	1612	97	<1.2	6.9	80	<1.2	<1.2	<1.2	<0.12
1-indanone	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.04

Düsseldorf-Flingern: HPLC-MS-MS data

Table 31: Data of quinoline compounds in the groundwater of the Düsseldorf-Flingern field site. Analyses were done using HPLC-MS-MS. Concentration [µg/L].

compound	19200				19201					19202				
	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m
quinoline	<0.006	<0.006	<0.006	<0.006	0.352	1.62	0.053	0.034	0.030	0.087	0.113	0.037	0.023	0.013
isoquinoline	0.126	0.110	0.085	0.081	7.74	2.50	0.201	0.148	0.128	0.425	0.865	0.172	0.104	0.116
1-methylisoquinoline	0.031	0.023	0.043	0.091	1.52	0.653	0.172	0.193	0.027	2.07	28.3	9.02	0.144	0.608
3-methylisoquinoline	0.051	0.038	0.041	0.047	1.13	0.842	0.036	0.038	0.041	0.451	3.30	0.089	0.052	0.032
2-methylquinoline	2.50	0.281	1.11	0.062	9.38	17.7	10.2	15.1	2.60	9.34	51.4	11.5	1.73	61.7
4-methylquinoline	0.028	0.015	0.005	0.004	0.386	0.962	0.051	0.012	0.016	0.072	0.339	0.028	0.015	0.016
6-methylquinoline	0.039	0.011	0.020	0.038	0.605	0.591	0.078	0.061	0.018	0.695	11.2	2.75	0.044	0.187
2(1H)-quinolinone	0.363	0.045	0.048	0.045	14.4	5.73	0.090	0.045	0.033	2.03	0.973	0.053	0.025	0.086
1(2H)-isoquinolinone	98.5	0.690	0.269	0.179	138	35.5	5.23	0.487	0.124	79.3	27.4	0.710	0.200	0.258
4(1H)-quinolinone	0.085	<0.008	0.024	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	0.035
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3,4-dihydro-2(1H)-quinolinone	0.319	0.011	<0.008	<0.008	5.43	0.422	0.106	0.037	0.026	0.367	0.158	<0.008	<0.008	0.030
3,4-dihydro1(2H)-isoquinolinone	0.022	0.008	<0.006	0.006	0.021	0.009	<0.006	<0.006	<0.006	0.007	0.006	<0.006	<0.006	<0.006
2-methyl-6-quinolinol	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	9.09	0.040	0.403	0.017	40.5	12.7	0.131	0.564	0.042	9.27	2.40	0.095	0.050	0.771
6-methoxyquinoline	<0.003	<0.003	<0.003	0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
1-methyl-2-quinolinone	0.397	0.094	0.077	0.027	0.807	0.232	0.162	0.098	0.077	0.617	0.351	0.116	0.052	0.052
2-methyl-4(1H)-quinolinone	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
2,4-dimethylquinoline	0.065	0.102	0.067	0.150	9.15	14.8	0.897	3.55	0.323	25.8	59.5	18.4	0.683	2.36
2,6-dimethylquinoline	0.021	0.027	0.024	0.045	0.461	1.47	0.072	2.54	0.120	7.69	33.4	11.2	0.223	0.197
1,2,3,4-tetrahydroquinoline	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
4-hydroxy-1-methyl-2(1H)-quinolinone	0.496	0.169	0.040	0.033	0.129	0.044	0.092	0.031	0.029	0.021	0.021	0.021	0.034	0.048

Table 31, continued (part 2). Concentration [µg/L].

compound	19203					19214				19185			
	6.5 m	7 m	8 m	9 m	10 m	6.5 m	8 m	9 m	10 m	7 m	8 m	9 m	10 m
quinoline	0.071	0.050	0.030	0.016	<0.006	0.050	<0.006	0.039	<0.006	0.047	0.012	0.030	0.007
isoquinoline	0.538	0.483	0.127	0.114	0.079	0.327	0.263	0.152	0.118	0.143	0.091	0.087	0.079
1-methylisoquinoline	1.908	14.965	3.692	2.166	0.016	0.305	0.038	0.048	0.012	6.208	2.013	5.653	3.068
3-methylisoquinoline	1.168	2.619	0.175	0.065	0.057	0.168	<0.008	0.041	0.039	0.175	0.068	0.065	0.124
2-methylquinoline	13.213	33.187	28.885	29.090	1.627	3.585	46.708	18.622	5.367	7.068	1.135	3.892	1.647
4-methylquinoline	0.296	0.238	0.022	0.010	<0.004	0.040	0.038	0.023	0.010	<0.004	0.010	0.012	0.013
6-methylquinoline	0.745	5.102	1.158	0.674	0.022	0.120	0.045	0.032	0.008	1.969	0.634	1.740	1.032
2(1H)-quinolinone	1.551	0.608	0.041	0.020	<0.010	1.836	3.065	0.088	0.025	0.340	<0.010	0.030	<0.010
1(2H)-isoquinolinone	16.687	32.798	0.509	0.283	0.157	18.605	51.979	0.825	0.183	4.156	0.228	0.238	0.134
4(1H)-quinolinone	<0.008	<0.008	<0.008	<0.008	0.043	0.063	0.054	<0.008	0.012	<0.008	<0.008	<0.008	0.026
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3,4-dihydro-2(1H)-quinolinone	0.174	0.128	0.027	<0.008	<0.008	0.155	0.652	0.034	0.017	0.043	<0.008	0.009	<0.008
3,4-dihydro1(2H)-isoquinolinone	0.006	<0.006	<0.006	<0.006	<0.006	0.007	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	0.006
2-methyl-6-quinolinol	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	5.206	3.189	0.085	0.080	0.008	2.739	4.470	0.090	0.087	0.489	<0.006	0.023	0.014
6-methoxyquinoline	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	0.006
1-methyl-2-quinolinone	0.122	0.306	0.087	0.027	<0.005	0.099	0.646	0.254	0.092	0.037	0.018	0.044	0.026
2-methyl-4(1H)-quinolinone	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
2,4-dimethylquinoline	31.474	33.532	30.818	27.929	0.116	5.253	0.116	0.045	0.039	44.651	46.402	33.313	18.604
2,6-dimethylquinoline	6.528	13.745	4.860	6.020	0.033	0.435	0.015	0.012	0.020	12.258	6.492	8.124	8.777
1,2,3,4-tetrahydroquinoline	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
4-hydroxy-1-methyl-2(1H)-quinolinone	0.014	0.012	0.013	0.059	0.041	0.025	0.352	0.410	0.058	0.016	0.009	0.029	0.053

Table 31, continued (part 3). Concentration [µg/L].

compound	19213				19186					19205				
	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m
quinoline	<0.006	<0.006	0.036	0.015	0.008	<0.006	0.007	<0.006	0.025	<0.006	<0.006	<0.006	0.024	0.009
isoquinoline	0.123	0.049	0.118	0.091	0.091	0.087	0.082	0.077	0.081	0.126	0.162	0.095	0.138	0.077
1-methylisoquinoline	2.26	16.0	11.5	0.369	0.114	0.078	0.117	0.089	0.608	<0.01	<0.01	<0.01	<0.01	<0.01
3-methylisoquinoline	<0.008	<0.008	<0.008	0.055	0.037	0.041	0.047	0.037	0.041	0.038	0.038	0.039	0.045	0.037
2-methylquinoline	19.7	70.7	47.7	3.93	3.93	2.68	0.096	0.145	0.578	6.08	41.0	125	41	0.254
4-methylquinoline	0.014	0.032	0.031	0.02	0.01	0.008	0.009	0.005	0.012	0.015	0.017	0.013	0.015	0.007
6-methylquinoline	0.693	5.25	3.70	0.131	0.04	0.026	0.043	0.03	0.192	0.019	0.034	0.215	0.17	0.036
2(1H)-quinolinone	0.011	0.019	0.016	0.02	0.008	<0.010	0.005	<0.010	<0.010	0.023	0.018	0.015	0.033	0.006
1(2H)-isoquinolinone	1.323	0.449	0.416	0.173	0.162	0.134	0.010	<0.034	<0.034	0.123	0.310	0.155	0.164	<0.034
4(1H)-quinolinone	0.034	<0.008	<0.008	<0.008	0.019	0.01	<0.008	<0.008	<0.008	0.017	<0.008	<0.008	0.041	0.037
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3,4-dihydro-2(1H)-quinolinone	<0.008	<0.008	<0.008	0.013	0.025	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	0.018	<0.008
3,4-dihydro1(2H)-isoquinolinone	<0.006	<0.006	<0.006	<0.006	0.009	<0.006	<0.006	<0.006	0.006	0.008	0.007	<0.006	<0.006	<0.006
2-methyl-6-quinolinol	0.012	0.02	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	0.019	0.077	0.052	0.058	0.067	0.018	0.008	<0.006	<0.006	0.054	<0.006	0.188	0.048	<0.006
6-methoxyquinoline	<0.003	<0.003	<0.003	<0.003	0.004	<0.003	0.004	<0.003	0.003	<0.003	<0.003	<0.003	<0.003	<0.003
1-methyl-2-quinolinone	0.175	0.22	0.164	0.048	0.005	0.007	0.007	<0.005	0.010	0.281	0.785	0.837	0.218	0.019
2-methyl-4(1H)-quinolinone	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
2,4-dimethylquinoline	17.9	47.3	42.3	0.530	0.09	0.053	0.182	2.57	7.49	0.142	0.034	0.084	0.245	0.060
2,6-dimethylquinoline	2.26	10.9	7.47	0.160	0.061	0.023	0.087	1.48	4.10	0.034	0.012	0.013	0.023	0.009
1,2,3,4-tetrahydroquinoline	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
4-hydroxy-1-methyl-2(1H)-quinolinone	0.028	0.018	0.026	0.072	0.014	0.01	0.009	0.009	0.031	0.041	0.062	0.062	0.042	0.035

Table 31, continued (part 4). Concentration [µg/L].

compound	19206				19064		
	7 m	8 m	9 m	10 m	12 m	13 m	10 m
quinoline	<0.006	0.020	0.015	<0.006	<0.006	<0.006	<0.006
isoquinoline	0.091	0.140	0.118	0.067	0.062	0.067	0.079
1-methylisoquinoline	0.045	0.042	0.012	<0.010	<0.010	<0.010	<0.01
3-methylisoquinoline	0.038	0.053	0.036	0.028	0.032	0.021	0.034
2-methylquinoline	0.041	0.391	0.053	0.019	0.016	0.014	0.028
4-methylquinoline	0.009	0.022	0.006	<0.004	<0.004	<0.004	0.006
6-methylquinoline	0.015	0.036	0.008	<0.004	<0.004	<0.004	<0.004
2(1H)-quinolinone	0.036	0.092	0.051	<0.01	<0.01	<0.01	0.013
1(2H)-isoquinolinone	0.066	0.371	0.138	<0.034	<0.034	<0.034	<0.034
4(1H)-quinolinone	<0.008	0.018	<0.008	<0.008	<0.008	<0.008	0.017
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3,4-dihydro-2(1H)-quinolinone	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008
3,4-dihydro1(2H)-isoquinolinone	0.007	0.010	<0.006	<0.006	<0.006	<0.006	<0.006
2-methyl-6-quinolinol	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	0.030	0.099	0.966	<0.006	<0.006	<0.006	<0.006
6-methoxyquinoline	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
1-methyl-2-quinolinone	0.087	0.194	0.118	0.012	0.006	0.004	0.017
2-methyl-4(1H)-quinolinone	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
2,4-dimethylquinoline	0.021	0.036	0.029	0.009	0.010	0.006	0.022
2,6-dimethylquinoline	0.007	0.009	0.007	<0.006	<0.006	<0.006	0.007
1,2,3,4-tetrahydroquinoline	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
4-hydroxy-1-methyl-2(1H)-quinolinone	0.122	0.612	0.058	0.034	0.023	0.014	0.025

Table 31, continued (part 5). Concentration [µg/L].

compound	19209				19210					19071*
	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	
quinoline	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
isoquinoline	0.086	0.098	0.154	0.098	0.096	0.153	0.084	0.082	0.083	0.021
1-methylisoquinoline	<0.010	0.017	0.012	<0.010	0.020	0.221	0.340	0.118	0.016	<0.010
3-methylisoquinoline	0.031	0.040	0.038	0.034	0.047	0.044	0.022	0.038	0.035	<0.008
2-methylquinoline	0.492	2.85	2.02	0.445	0.279	29.1	51.6	0.078	0.029	0.054
4-methylquinoline	0.008	0.023	0.018	0.005	0.007	0.015	0.008	0.004	0.006	<0.004
6-methylquinoline	<0.004	0.024	0.019	<0.004	0.010	0.069	0.102	0.036	0.007	<0.004
2(1H)-quinolinone	0.228	0.891	0.047	0.032	0.027	0.041	0.011	<0.01	0.003	0.002
1(2H)-isoquinolinone	0.343	3.95	3.17	0.152	0.134	0.332	0.051	<0.034	<0.034	0.017
4(1H)-quinolinone	0.033	0.035	<0.008	<0.008	0.018	0.071	0.010	0.010	<0.008	<0.008
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3(2H)-isoquinolinone	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3,4-dihydro-2(1H)-quinolinone	0.205	1.13	0.043	0.016	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008
3,4-dihydro1(2H)-isoquinolinone	0.014	0.015	0.006	<0.006	0.009	0.010	<0.006	<0.006	<0.006	0.004
2-methyl-6-quinolinol	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	9.24	18.0	0.262	0.046	0.154	0.215	<0.006	<0.006	<0.006	0.008
6-methoxyquinoline	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
1-methyl-2-quinolinone	0.349	0.730	0.660	0.151	0.105	0.976	0.067	0.014	0.009	0.023
2-methyl-4(1H)-quinolinone	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
2,4-dimethylquinoline	0.015	0.017	0.027	0.025	0.060	0.146	0.055	0.043	0.058	<0.006
2,6-dimethylquinoline	<0.006	0.006	0.009	0.013	0.021	0.034	0.013	0.010	0.012	<0.006
1,2,3,4-tetrahydroquinoline	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.005
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
4-hydroxy-1-methyl-2(1H)-quinolinone	0.401	0.700	0.847	0.091	0.038	0.551	0.068	0.040	0.023	<0.006

* for sample 19071, limits of detection are about a factor 10 lower because of 10 L groundwater sample enriched.

Düsseldorf-Flingern: Distribution of compounds within the field site

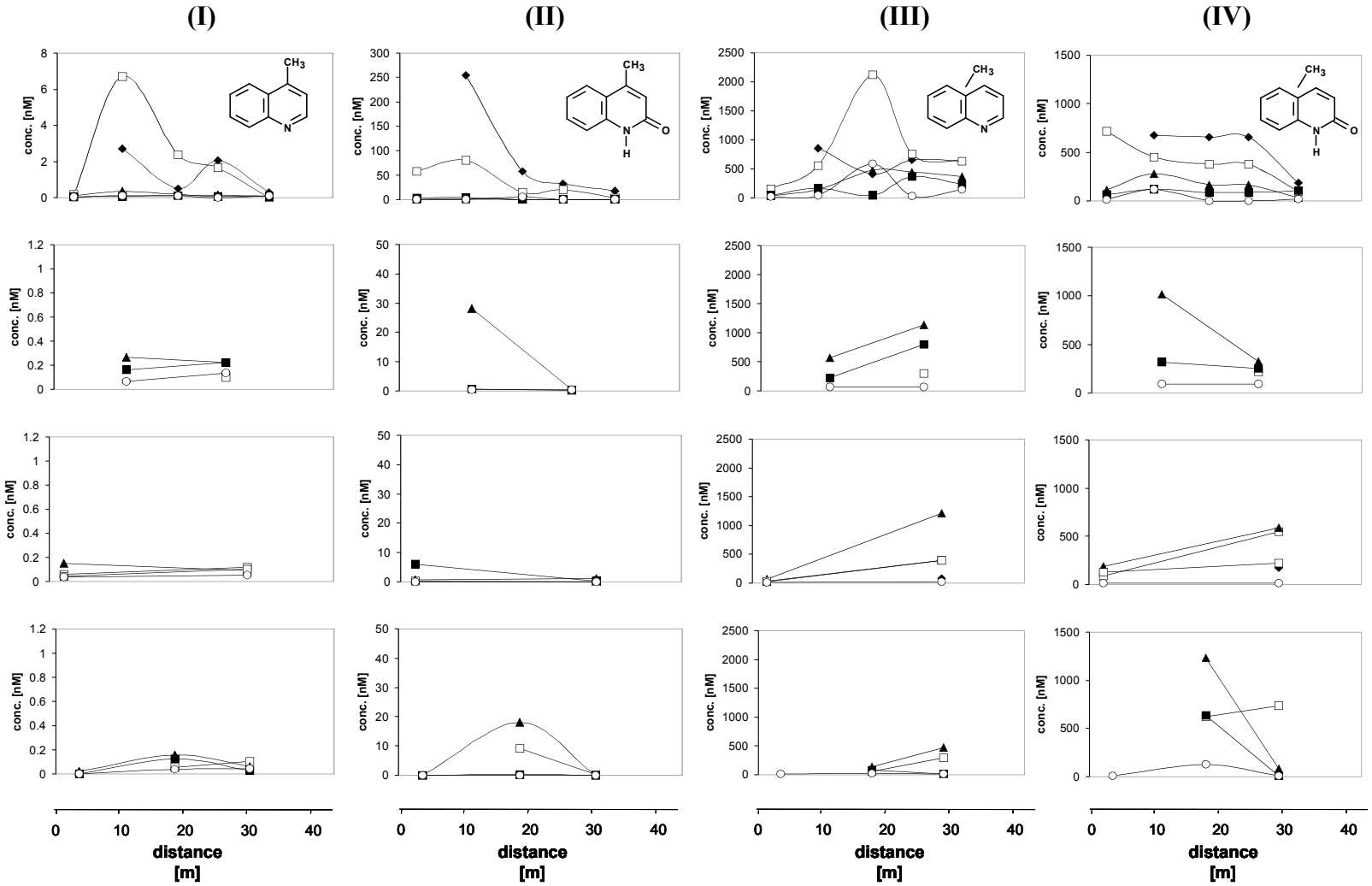


Figure 50: (I) 4-methylquinoline, (II) 4-methyl-2(1H)-quinolinone, (III) Σ methylquinoline, (IV) Σ methyl-2(1H)-quinolinone. The levels analysed are (◆) = 6.5 m, (□) = 7 m, (▲) = 8 m, (■) = 9 m, (○) = 10 m below top ground surface. Methylquinolines: 1 nM = 143 ng/L; methyl-2(1H)-quinolinones 1 nM = 159 ng/L.

7.4.3 Wülknitz: Field data

Wülknitz: HPLC-DAD data

Table 32: HPLC-DAD data of NSO-heterocyclic and homocyclic compounds in the groundwater of the Wülknitz field site.

compound	20/05			24/05			21/05	12/98		13/01		06/98		14/01	
	4-8 m	13-17 m	26-30 m	4-8 m	12-16 m	26-30 m	10-14 m	19-21 m	27-29 m	13-15 m	26-30 m	20-22 m	29-30 m	16-20 m	27-31 m
carbazole	<0.4	<0.4	48.0	25.0	169.7	34.1	41.1	8.0	<0.4	37.1	<0.4	<0.4	<0.4	<0.4	<0.4
phenanthridinone	<0.4	<0.4	<0.4	46.6	<0.4	17.1	68.5	<0.4	<0.4	58.8	<0.4	<0.4	<0.4	<0.4	<0.4
acridine	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
acridinone	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4	<2.4
benzothiophene	1046.8	715.3	73.1	86.8	587.2	63.9	195.4	10.7	3.5	241.5	43.2	70.8	8.0	11.8	32.9
3-methylbenzothiophene	132.9	115.6	45.3	19.4	137.9	32.0	56.8	1.1	<0.8	81.3	<0.8	11.0	<0.8	<0.8	<0.8
methylbenzothiophene isomer	141.7	122.6	33.5	28.7	129.9	19.3	6.5	<0.8	<0.8	<0.8	<0.8	8.9	<0.8	<0.8	<0.8
dibenzothiophene	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
benzofuran	540.0	544.9	82.2	<0.4	150.2	12.9	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-methylbenzofuran	436.4	446.9	17.6	133.1	482.6	29.8	164.9	12.3	1.7	329.9	33.9	74.0	5.9	11.7	27.0
2,3-dimethylbenzofuran	159.3	61.5	63.7	16.5	101.9	17.0	36.5	<0.4	<0.4	46.7	<0.4	6.0	<0.4	<0.4	<0.4
dimethylbenzofuran isomer	75.5	62.3	33.9	17.7	92.5	14.3	22.6	0.8	<0.4	45.5	<0.4	7.0	<0.4	1.0	1.0
dibenzofuran	<0.4	157.9	151.0	114.9	284.7	240.9	35.4	4.5	<0.4	81.6	<0.4	<0.4	<0.4	<0.4	<0.4
naphthalene	735.4	2188.2	506.7	156.9	1012.8	489.9	35.0	3.9	4.1	193.7	100.0	4.5	0.6	1.4	1.9
1-methylnaphthalene	558.3	597.3	217.5	74.7	595.2	272.9	<0.4	2.7	2.4	80.7	<0.4	3.0	<0.4	1.9	3.5
2-methylnaphthalene	<0.4	205.4	329.7	19.9	305.5	303.6	12.9	0.6	1.2	<0.4	<0.4	<0.4	<0.4	1.0	0.5
1,3-dimethylnaphthalene	79.7	<0.4	<0.4	20.1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
2-naphthol	<0.4	516.3	<0.4	<0.4	170.7	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
1-naphthol	308.4	383.1	25.5	<0.4	148.7	10.2	29.9	<0.4	<0.4	31.0	17.3	<0.4	<0.4	1.9	<0.4
2-naphthoic acid	192.0	103.0	60.3	<1.2	118.7	<1.2	<1.2	<1.2	<1.2	<1.2	21.4	<1.2	<1.2	<1.2	<1.2
fluorene	<0.6	133.5	137.4	33.7	324.5	211.6	10.5	2.1	<0.6	31.4	<0.6	<0.6	<0.6	<0.6	<0.6
acenaphthenol	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
acenaphthene	410.9	650.9	208.2	118.7	556.2	241.3	197.4	13.4	3.7	196.8	<1.2	19.7	<1.2	6.5	<1.2
indene	667.2	374.2	115.3	21.2	255.0	35.0	35.3	<0.4	<0.4	45.9	9.9	<0.4	<0.4	<0.4	<0.4
indan	314.6	591.0	49.3	97.2	627.8	53.2	157.7	12.1	4.3	302.4	52.4	79.4	13.9	20.0	43.8
1-indanone	383.4	245.1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

Wülknitz: HPLC-MS-MS data

Table 33: Data of quinoline compounds in the groundwater of the Wülknitz field site. Analyses were done using HPLC-MS-MS.

compound	20/05			24/05			21/05	12/98		13/01		06/98		14/01	
	4-8 m	13-17 m	26-30 m	4-8 m	12-16 m	26-30 m	10-14 m	19-21 m	27-29 m	13-15 m	26-30 m	20-22 m	29-30 m	16-20 m	27-31 m
quinoline	3.574	13.014	4.836	<0.006	<0.006	0.091	0.723	<0.006	<0.006	<0.006	0.034	<0.006	0.003	<0.006	<0.006
2(1H)-quinolinone	12.412	42.620	21.200	0.732	60.634	0.300	3.790	0.012	<0.010	2.471	0.096	0.052	<0.010	0.013	0.015
3,4-dihydro-2(1H)-quinolinone	68.042	14.013	0.325	<0.008	8.864	0.263	0.332	<0.008	<0.008	1.210	0.030	<0.008	<0.008	<0.008	<0.008
isoquinoline	6.732	12.917	2.061	0.506	0.733	0.142	2.022	0.005	0.007	0.442	0.071	0.108	<0.014	<0.014	0.006
1(2H)-isoquinolinone	2617.459	1834.397	7.541	17.329	978.828	27.987	11.528	0.055	0.047	144.287	10.803	<0.034	0.039	<0.034	0.110
3,4-dihydro-1(2H)-isoquinolinone	189.112	19.582	0.357	0.927	1.097	0.553	0.104	0.080	0.013	0.834	0.286	0.382	0.069	0.239	0.165
4(1H)-quinolinone	<0.008	<0.008	0.044	0.343	<0.008	0.041	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008	0.011	<0.008
5(1H)-quinolinone	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
6-quinolinol	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
7-isoquinolinol	11.485	8.830	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
3(2H)-isoquinolinone	12.242	<0.004	0.101	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	0.071	<0.004	<0.004	<0.004	0.016
4-methyl-2,7-quinolinol	13.326	16.044	0.166	<0.005	<0.005	0.491	0.054	<0.005	<0.005	<0.005	0.052	<0.005	<0.005	<0.005	0.021
2-methyl-6-quinolinol	12.636	15.685	0.193	<0.003	<0.003	<0.003	0.047	<0.003	<0.003	<0.003	0.056	<0.003	<0.003	<0.003	<0.003
4-methyl-2(1H)-quinolinone	791.142	861.000	3.475	3.336	242.757	0.885	4.157	0.014	0.022	36.850	0.187	0.182	<0.006	0.073	0.056
6-methoxyquinoline	3.690	4.892	0.062	0.161	0.203	0.064	0.033	<0.003	<0.003	0.191	0.030	0.027	<0.003	<0.003	<0.003
1-methyl-2-quinolinone	15.362	14.931	0.175	1.025	2.584	0.482	0.106	0.015	<0.005	0.877	0.069	0.267	<0.005	0.062	<0.005
2-methyl-4(1H)-quinolinone	115.900	6.554	0.060	2.396	1.318	17.292	<0.006	0.017	<0.006	4.480	<0.006	0.133	0.009	0.220	<0.006
2-methylquinoline	1.938	25.292	5.329	0.211	22.583	10.275	6.323	<0.006	0.011	0.508	0.013	<0.006	<0.006	<0.006	<0.006
4-methylquinoline	32.438	43.047	2.887	5.172	4.039	0.167	2.928	<0.004	0.009	2.866	0.087	0.074	<0.004	<0.004	<0.004
6-methylquinoline	5.872	14.179	1.514	0.433	0.660	0.210	1.594	0.005	0.006	0.369	0.090	0.087	<0.004	<0.004	<0.004
1-methylisoquinoline	11.092	13.985	0.306	0.414	0.824	0.183	0.365	<0.010	<0.010	0.388	0.083	0.087	<0.010	<0.010	<0.010
3-methylisoquinoline	4.098	11.649	0.962	0.229	0.880	0.169	1.145	<0.008	<0.008	0.290	0.070	0.072	<0.008	<0.008	0.009
2,4-dimethylquinoline	65.466	10.013	0.456	0.316	2.739	0.114	0.491	<0.006	<0.006	0.400	0.054	0.045	<0.006	<0.006	<0.006
2,6-dimethylquinoline	52.021	7.721	0.607	0.379	2.106	0.087	0.566	<0.006	<0.006	0.314	0.044	0.041	<0.006	<0.006	<0.006
1,2,3,4-tetrahydroquinoline	4.032	3.389	0.222	<0.010	0.238	0.098	0.078	<0.010	<0.010	<0.010	0.084	0.054	<0.010	<0.010	<0.010

Wülknitz: Distribution of compounds within the field site

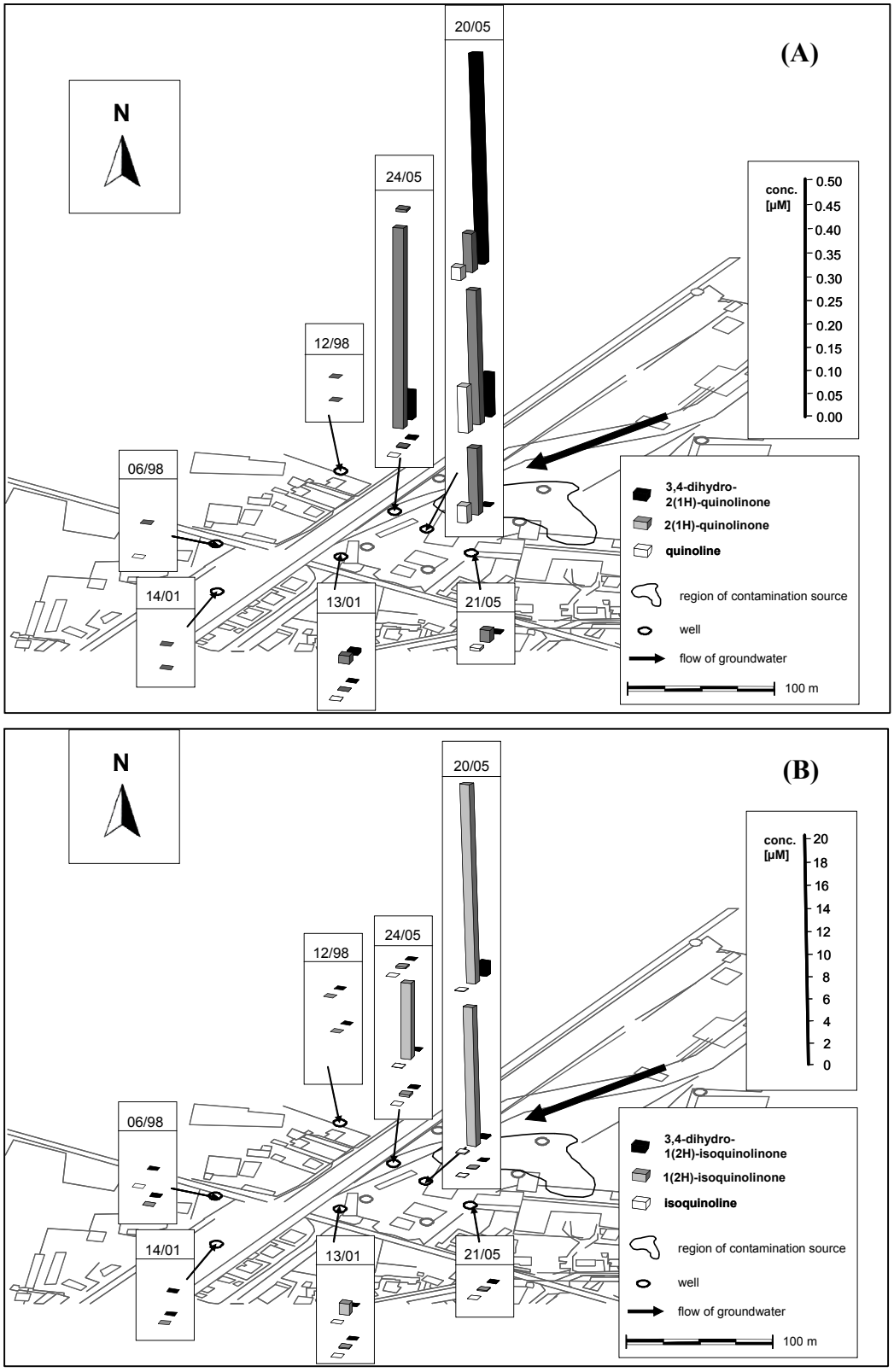


Figure 51: Distribution of quinoline, 2(1H)-quinolinone and 3,4-dihydro-2(1H)-quinolinone (A); isoquinoline, 1(2H)-isoquinolinone and 3,4-dihydro-2(1H)-isoquinolinone (B) in groundwater of the Wülknitz site. The arrow marks direction of groundwater flow; bars mark the presence and concentration of analysed compounds in three different depth. Quinoline and isoquinoline: 1 µM = 129 µg/L; 2(1H)-quinolinone and 1(2H)-isoquinolinone 1µM = 145 µg/L; 3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone: 1 µM = 147 µg/L.

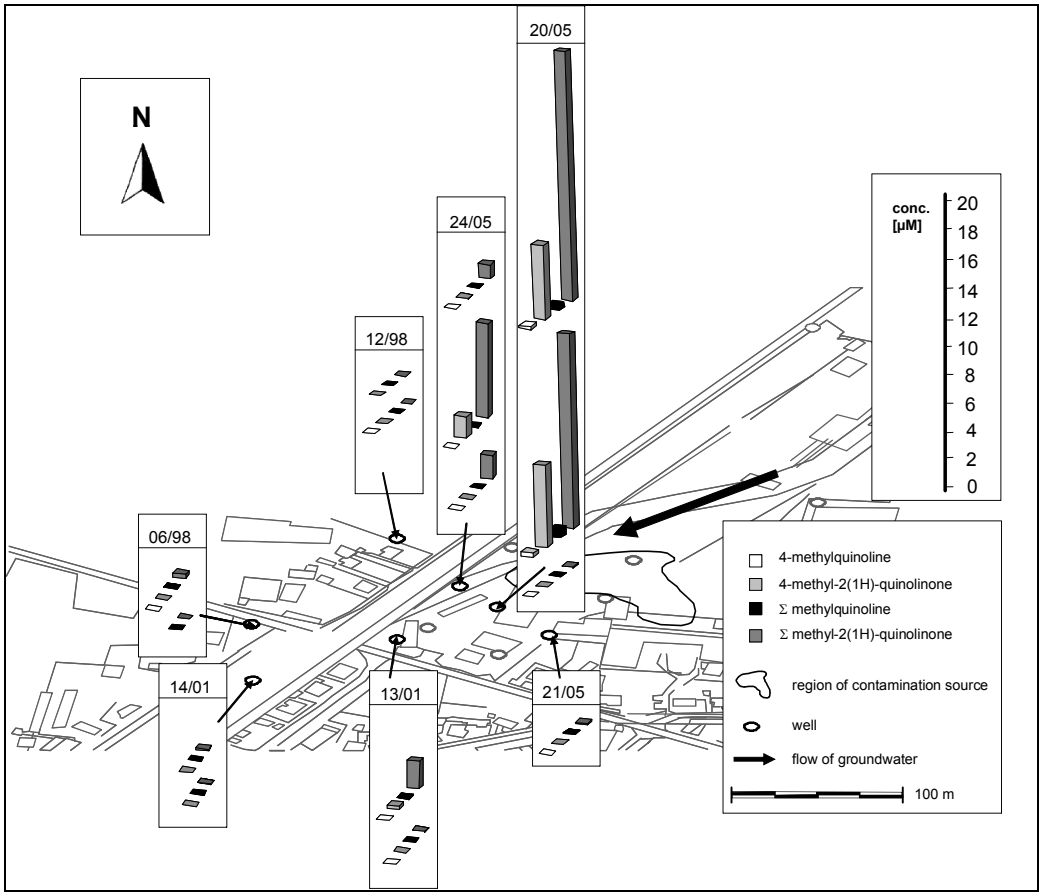


Figure 52: Distribution of 4-methylquinoline, 4-methyl-2(1H)-quinolinone, Σ methylquinolines, Σ methyl-2(1H)-quinolinone in groundwater of the Wülknitz site. The arrow marks direction of groundwater flow; bars mark the presence and concentration of analysed compounds in three different depths. Methylquinolines: 1 µM = 143 µg/L; methyl-2(1H)-quinolinones: 1 µM = 159 µg/L.

7.5 Site-directed analyses: Indications for microbial degradative potential

Anaerobic microcosms with groundwater from the Wülknitz site

Table 34: Concentrations at the beginning of the degradation experiments in the Wülknitz groundwater

compound	concentration [µg/L]				
	IW1	IW5	13/01	IW2	1/97
N-Heterocycles					
quinoline	1166	<0.06	<0.06	10.4	0.573
isoquinoline	167	8.22	0.961	28.8	15.6
1-methylisoquinoline	89.6	2.92	4.50	24.1	2.57
3-methylisoquinoline	118	1.40	1.40	12.2	2.07
2-methylquinoline	1780	<0.04	0.419	10.7	1.56
4-methylquinoline	861	6.64	0.045	86.8	4.49
6-methylquinoline	89.6	1.50	1.390	14.8	1.22
2(1H)-quinolinone	8096	11.0	0.388	3377	1.46
1(2H)-isoquinolinone	3344	661	3.67	1173	122
4(1H)-quinolinone	5.28	0.26	0.045	2.06	0.136
5(1H)-quinolinone	53.1	<0.07	<0.07	<0.07	<0.07
7-isoquinolinol	26.8	<0.04	<0.04	<0.04	<0.04
3,4-dihydro-2(1H)-quinolinone	84.1	1.49	0.25	32.4	1.19
3,4-dihydro-1(2H)-isoquinolinone	29.0	3.27	1.15	23.3	1.70
2-methyl-6-quinolinol	34.2	1.40	<0.03	28.2	1.42
4-methyl-2(1H)-quinolinone	802	37	1.51	214.	45.11
6-methoxyquinoline	13.9	0.471	<0.03	5.39	0.432
1-methyl-2-quinolinone	28.2	2.22	0.514	13.3	1.16
2-methyl-4(1H)-quinolinone	19.1	36.4	1.43	5.61	0.858
2,4-dimethylquinoline	673	0.876	0.03	12.6	0.727
2,6-dimethylquinoline	78.9	0.775	<0.06	10.5	0.683
7-hydroxy-4-methyl-2(1H)-quinolinone	24.1	1.78	<0.05	13.8	0.722
1,2,3,4-tetrahydroquinoline	8.30	0.731	<0.13	11.3	0.489
carbazole	432	68.9	83.4	51.4	61.2
6(5H)-phenanthridinone	734	202	161	<4	43.8
S-Heterocycles					
benzothiophene	976	222	171	266	221
O-Heterocycles					
benzofuran	790	70.5	<4	202	109
2-methylbenzofuran	367	205	196	187	163
2,3-dimethylbenzofuran	47	22.9	34.9	31.2	
dibenzofuran	137	46.6	68.9	53.8	36.6
PAHs/Homocycles					
naphthalene	3991	83.7	30.9	727	1246
fluorene	89	30.0	34.1	38.9	28.3
acenaphthene	248	126	174	99.4	94.3
1-methylnaphthalene	310	65.5	<4	134	127
2-methylnaphthalene	424	<4	<4	45.8	112
1,3-dimethylnaphthalene	38	29.7	25.6	35.1	27.1
indene	475	66.3	<4	137	<4
indan	448	255	215	258	88.2
1-indanone	417	<4	<4	192	61.0
1-naphthol	<4	<4	82.8	<4	<4
2-naphthol	1268	<4	<4	<4	<4

100 mL groundwater sample were enriched. Results of sulfate- and iron-reducing microcosms were averaged.

Table 35: Concentrations of electron acceptor in the microcosms with groundwater of the Wülknitz site.

well	conditions	Fe^{2+} [mg/L]		NO_3^- [mg/L]		SO_4^{2-} [mg/L]	
		biotic microcosm	abiotic control	biotic microcosm	abiotic control	biotic microcosm	abiotic control
IW1	sulfate-reducing	0.28	1.28	4.69	177.33	80.17	119.84
	iron-reducing	5.60	<0.10	1.28	176.45	101.20	120.49
IW2	sulfate-reducing	<0.10	0.72	3.91	179.02	239.64	245.27
	iron-reducing	5.06	<0.10	1.46	179.05	239.59	239.21
IW5	sulfate-reducing	<0.10	2.42	3.17	174.98	35.33	51.64
	iron-reducing	<0.10	<0.10	6.90	176.75	53.44	50.93
13/01	sulfate-reducing	<0.10	29.10	3.05	180.50	8.88	104.24
	iron-reducing	1.72	0.94	1.26	171.01	45.09	100.50
1/97	sulfate-reducing	<0.10	10.20	2.61	183.64	334.56	360.47
	iron-reducing	<0.10	<0.10	11.46	190.25	358.84	363.36

High-resolution well analysis at the Düsseldorf site

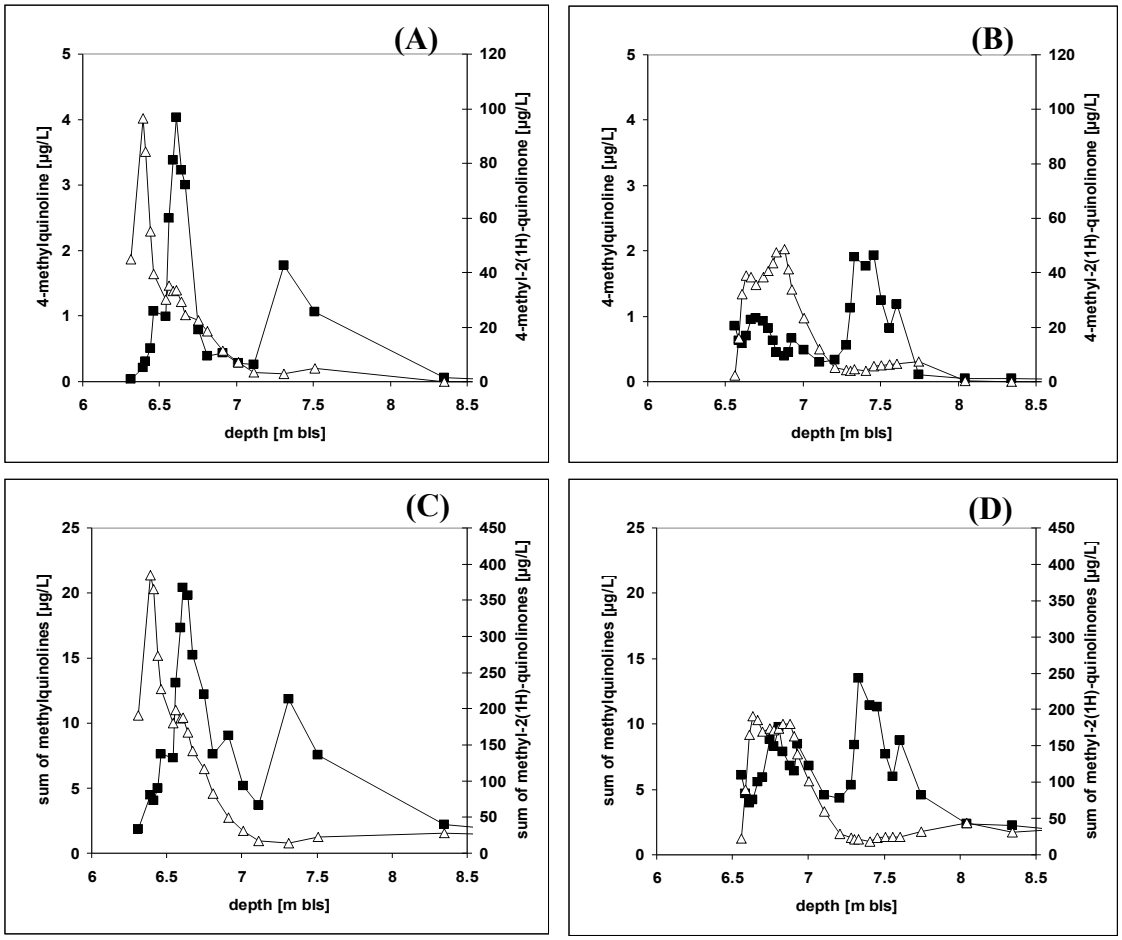


Figure 53: 4-Methylquinoline and 4-methyl-2(1H)-quinolinone distribution in the high-resolution well in Düsseldorf-Flingern (A) in August, (B) in February. Sum of methylquinolines and methyl-2(1H)-quinolinones distribution (C) in August, (D) in February. Legend: corresponding methylquinoline (■) and methyl-2(1H)-quinolinone (△).

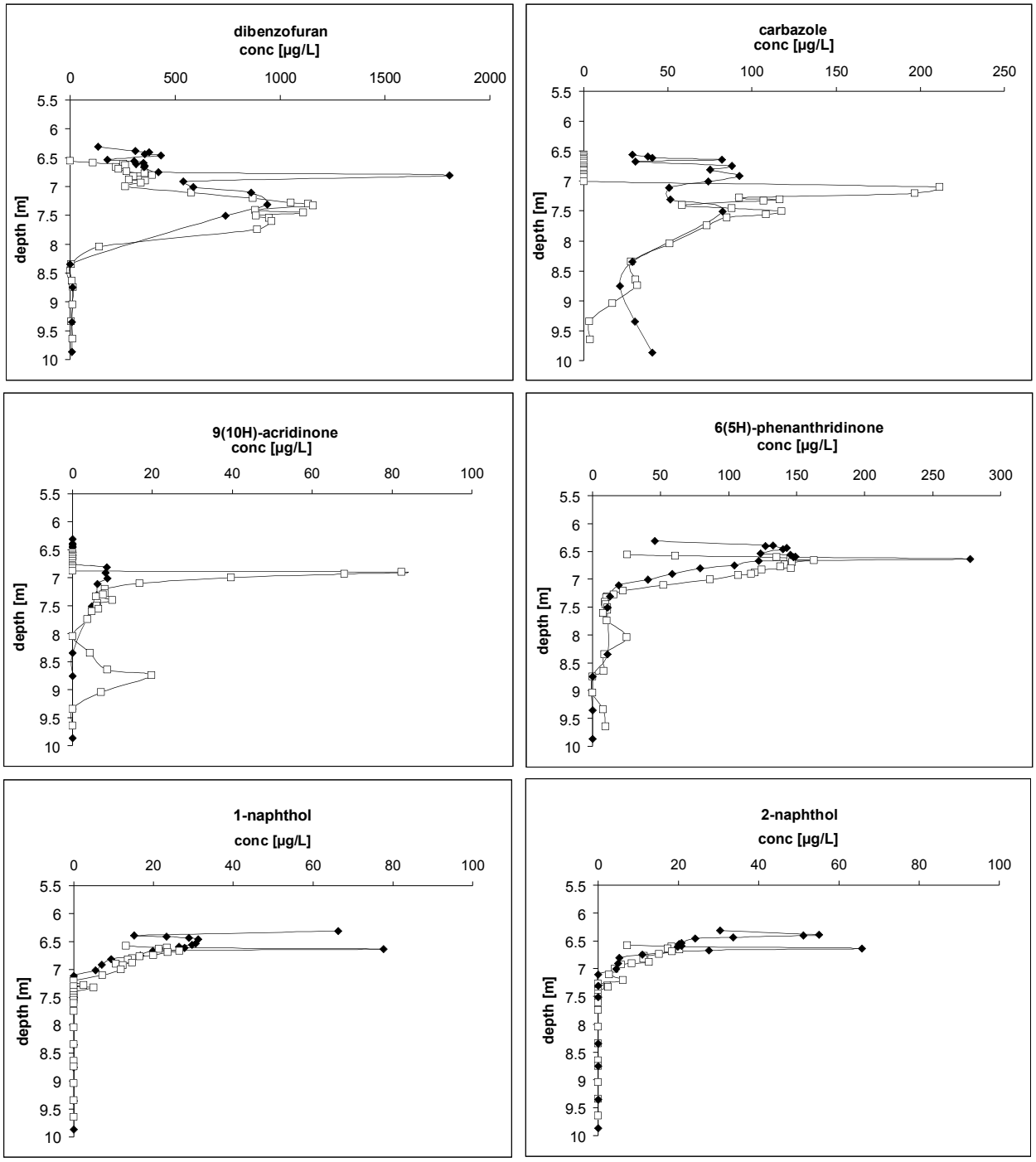


Figure 54: Vertical gradients of selected heterocyclic and homocyclic compounds in the high-resolution well in Düsseldorf-Flingern. Legend: August 2006 (◆) and February 2007 (□).

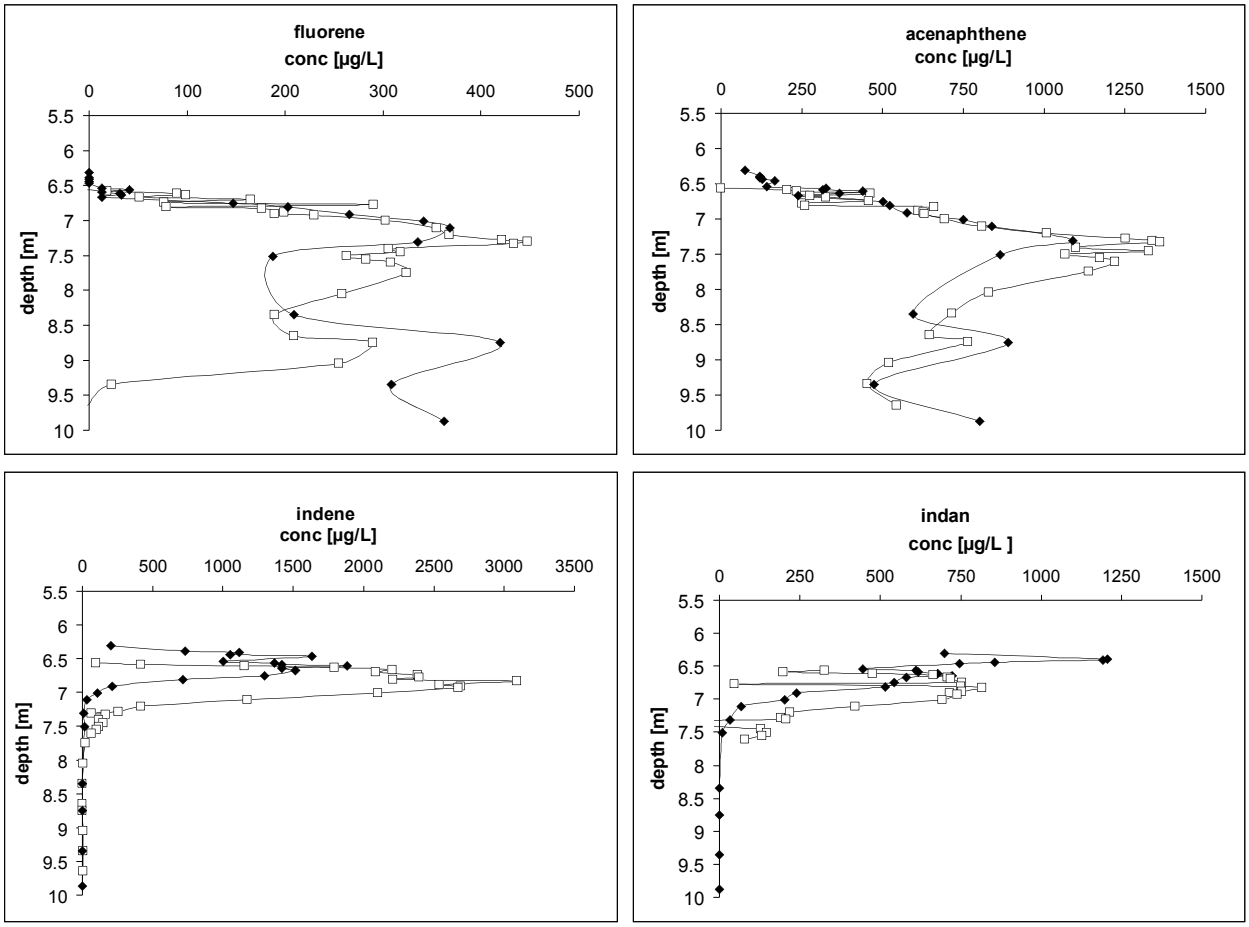


Figure 55: Vertical gradients of selected heterocyclic and homocyclic compounds in the high-resolution well in Düsseldorf-Flingern. Legend: August 2006 (◆) and February 2007 (□).

7.6. Hydroxylated quinolines: important characteristics and fate assessment

7.6.1 Toxicity of quinolines and their metabolites

Table 36: Effects of quinoline compounds in the *umuC* test given as NOEC and LOEC (mg/L).

compound	*umuC* test			
	- S9		+ S9	
	LOEC	NOEC	LOEC	NOEC
quinoline*	-	132	-	132
isoquinoline*	-	38	-	38
2-methylquinoline	-	139	-	139
3-methylquinoline	-	136	136	68
4-methylquinoline	139	70	139	70
6-methylquinoline	-	139	-	139
7-methylquinoline	-	133	-	133
8-methylquinoline	-	134	-	134
2(1H)-quinolinone*	-	132	-	132
1(2H)-isoquinolinone*	-	132	-	132
3,4-dihydro-2(1H)-quinolinone*	-	132	-	132
3,4-dihydro-1(2H)-*	-	132	-	132
4-methyl-2(1H)-quinolinone	67	33	133	67
2-methyl-6-quinolinol	133	67	-	133
2-methyl-4(1H)-quinolinone	-	133	-	133
methyl-3,4-dihydro-2(1H)-quinolinone mixture**	-	133	-	133

*data were taken from the Diploma thesis of Neuwoehner (2006) as well as Neuwoehner et al., 2007.

Table 37: Effects of quinoline compounds in the Ames fluctuation test given as NOEC and LOEC (mg/L).

compound	Ames fluctuation test							
	TA 98				TA 100			
	- S9		+ S9		- S9		+ S9	
	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC	NOEC
quinoline*	-	160	60	80	-	160	60	20
isoquinoline*	-	45	-	45	-	45	-	45
2-methylquinoline	-	167	-	167	-	160	52	21
3-methylquinoline	-	163	-	156	-	163	20	2
4-methylquinoline	-	167	21	2	-	167	2	1
6-methylquinoline	-	167	42	21	-	167	21	2
7-methylquinoline	-	160	-	153	40	20	2	1
8-methylquinoline	-	160	-	154	-	160	100	50
2(1H)-quinolinone*	-	160	-	153	-	160	-	160
1(2H)-isoquinolinone*	-	160	-	153	-	160	-	153
3,4-dihydro-2(1H)-quinolinone*	-	160	-	153	-	160	-	153
3,4-dihydro-1(2H)-isoquinolinone*	-	160	-	153	-	160	-	153
4-methyl-2(1H)-quinolinone	100	80	-	153	-	160	20	2
2-methyl-6-quinolinol	-	160	-	153	-	160	-	153
2-methyl-4(1H)-quinolinone	-	160	-	153	-	160	-	153
methyl-3,4-dihydro-2(1H)-quinolinone mixture**	-	160	-	153	-	160	-	153

*data were taken from the Diploma thesis of Neuwoehner (2006) as well as Neuwoehner et al., 2007.

7.6.2 Anaerobic degradation of quinoline compounds

Theoretical considerations on microbial degradation of quinolines

The changes in Gibbs free energy ($\Delta G^{o'}_{(aq)}$) for complete transformation of quinoline compounds under sulfate-, iron- and nitrate-reducing conditions were calculated. Free energy of formation ($\Delta G^{o}_{f\,(aq)}$ [kJ/mol]) of quinoline compounds used in the presented equations were derived from $\Delta G^{o}_{f\,(gas)}$ values for the hypothetical gaseous state by conversion via the real vapour pressure at 298 K. $\Delta G^{o}_{f\,(gas)}$ were derived from ChemDraw® Ultra-Program using Joback´s fragmentation method. For conversion into aqueous state the following formula was used:

$$\Delta G^{o'}_{f\,(aq)} = \Delta G^{o}_{f\,(gas)} + RT \ln p/p_{\circ}$$

where R is the universal gas constant (8.3124 J/K × mol), T the temperature (298 K), p the vapour pressure of compound and p_o the pressure under standard conditions. Values for p were taken from experimental data of the MPBPWIN-program or calculated data using the mean of the Grain and Antoine method (U.S. EPA. 2000, Episuite).

Calculations of the changes in Gibbs free energy at standard conditions are done according to the following equation:

$$\Delta G^{o'}_{(aq)} = \Sigma\, \Delta\, G^{o'}_{f\,(aq)}\ (\text{products}) - \Sigma\ \chi\ G^{o'}_{f\,(aq)}\ (\text{reactants})$$

The results of these changes in Gibbs free energies are presented in Table 38.

Table 38: Stoichiometry and energetics of mineralization of quinoline compounds under nitrate-, iron- and sulfate-reducing conditions.

conditions	change in free energy $\Delta G^{o'}_{(aq)}$
sulfate-reducing conditions	**kJ/mol SO_4^{2-}**
quinoline/isoquinoline $C_9H_7N + 5\ SO_4^{2-} + 2\ H^+ + 7\ H_2O \rightarrow 9HCO_3^- + 5\ H_2S + NH_4^+$	-67.1
methylquinolines (2-, 3-, 4-, 6-, 7-, 8-) $C_9H_7N(CH_3) + 5.75\ SO_4^{2-} + 2.5\ H^+ + 7\ H_2O \rightarrow 10\ HCO_3^- + 5.75\ H_2S + NH_4^+$	-62.3
2(1H)-quinolinone and 1(2H)-isoquinolinone $C_9H_7N(OH) + 4.75\ SO_4^{2-} + 1.5\ H^+ + 7\ H_2O \rightarrow 9\ HCO_3^- + 4.75\ H_2S + NH_4^+$	-81.6
3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone $C_9H_8N(OH) + 5\ SO_4^{2-} + 2\ H^+ + 6\ H_2O \rightarrow 9\ HCO_3^- + 5\ H_2S + NH_4^+$	-80.5
2,4- and 2,6-dimethylquinoline $C_9H_5N(CH_3)_2 + 6.5\ SO_4^{2-} + 3\ H^+ + 7\ H_2O \rightarrow 11\ HCO_3^- + 6.5\ H_2S + NH_4^+$	-59.3
methylquinolinones $C_9H_5(CH_3)N(OH) + 5.5\ SO_4^{2-} + 2\ H^+ + 7\ H_2O \rightarrow 10\ HCO_3^- + 5.5\ H_2S + NH_4^+$	-43.2
iron-reducing conditions	**kJ/mol Fe(III)**
quinoline/isoquinoline $C_9H_7N + 40\ Fe(OH)_3 + 31\ HCO_3^- + 32\ H^+ \rightarrow 40\ FeCO_3 + NH_4^+ + 93\ H_2O$ $C_9H_7N + \alpha\text{-}FeO(OH) + 31\ HCO_3^- + 32\ H^+ \rightarrow 40\ FeCO_3 + NH_4^+ + 53\ H_2O$	-41.9 -15.1
methylquinolines (2-, 3-, 4-, 6-, 7-, 8-) $C_9H_7N(CH_3) + 46\ Fe(OH)_3 + 36\ HCO_3^- + 37\ H^+ \rightarrow 46\ FeCO_3 + NH_4^+ + 108\ H_2O$	-41.3
2(1H)-quinolinone and 1(2H)-isoquinolinone $C_9H_7N(OH) + 38\ Fe(OH)_3 + 29\ HCO_3^- + 30\ H^+ \rightarrow 38\ FeCO_3 + NH_4^+ + 88\ H_2O$	-43.7
3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone $C_9H_8N(OH) + 40\ Fe(OH)_3 + 31\ HCO_3^- + 32\ H^+ \rightarrow 40\ FeCO_3 + NH_4^+ + 94\ H_2O$	-43.6
2,4- and 2,6-dimethylquinoline $C_9H_5N(CH_3)_2 + 52\ Fe(OH)_3 + 41\ HCO_3^- + 42\ H^+ \rightarrow 52\ FeCO_3 + NH_4^+ + 123\ H_2O$	-40.9
methyl(iso)quinolinones $C_9H_5(CH_3)N(OH) + 44\ Fe(OH)_3 + 34\ HCO_3^- + 35\ H^+ \rightarrow 44\ FeCO_3 + NH_4^+ + 103\ H_2O$	-43.2

Table 38, continued (part 2).

conditions	change in free energy $\Delta G^{o'}_{(aq)}$
nitrate reducing-conditions	**kJ/mol NO_3^{2-}**
quinoline/isoquinoline $C_9H_7N + 8\ NO_3^- + 3\ H_2O \rightarrow 9\ HCO_3^- + NH_4^+ + 4\ N_2$	-581.2
methylquinolines (2-, 3-, 4-, 6-, 7-, 8-) $C_9H_7N(CH_3) + 9.2\ NO_3^- + 2.4\ H_2O + 0.2\ H^+ \rightarrow 10\ HCO_3^- + NH_4^+ + 4.6\ N_2$	-588.0
2(1H)-quinolinone and 1(2H)-isoquinolinone $C_9H_6N(OH) + 7.6\ NO_3^- + 3.2\ H_2O \rightarrow 9\ HCO_3^- + NH_4^+ + 3.9\ N_2 + 0.4\ H^+$	-627.7
3,4-dihydro-2(1H)-quinolinone and 3,4-dihydro-1(2H)-isoquinolinone $C_9H_8N(OH) + 8\ NO_3^- + 2\ H_2O \rightarrow 9\ HCO_3^- + NH_4^+ + 4\ N_2$	-589.6
2,4- and 2,6-dimethylquinoline $C_9H_5N(CH_3)_2 + 10.4\ NO_3^- + 1.8\ H_2O + 0.4\ H^+ \rightarrow 11\ HCO_3^- + NH_4^+ + 5.2\ N_2$	-576.4
methyl(iso)quinolinones $C_9H_5(CH_3)N(OH) + 8.8\ NO_3^- + 2.6\ H_2O \rightarrow 10\ HCO_3^- + NH_4^+ + 4.4\ N_2 + 0.2\ H^+$	-588.0

The following free energies of formation ($G^o_{f\ (aq)}$ [kJ/mol]) of organic compounds were used: quinoline: 297.6; isoquinoline: 298.0; 2-methylquinoline: 291.8; 3-methylquinoline: 293.1; 4-methylquinoline: 290.8; 6-methylquinoline: 290.9; 7-methylquinoline: 290.9; 8-methylquinoline: 291.8, 2,4-dimethylquinoline: 290.2; 2,6-dimethylquinoline: 288.7; 2(1H)-quinolinone 151.3, 1(2H)-isoquinolinone: 150.4; 3,4-dihydroquinolinone: 128.3; 3,4-dihydroisoquinolinone: 127.2; 4-methyl-2(1H)-quinolinone: 162.3; 2-methyl-4(1H)-quinolinone: 142.7.

For inorganic compounds free energies of formation used for the calculations were as follows: $Fe(OH)_3$: -699 kJ/mol; α-FeO(OH): -488.6; NH_4^+ : -79.37; H^+: -39.83; H_2O: -237.17; N_2: 0; HCO_3^-: -586.85; $FeCO_3$: -666.7; H_2S: -27.87; NO_3^-:- 111.34; SO_4^{2-}: -744.6 (Spormann and Widdel, 2000; Madigan et al., 2000). The comparison of the equations of quinoline degradation under iron-reducing conditions with amorphous iron hydroxide or goethite show some differences in energetics. Geothite is the energetically less favourable electron acceptor.

Anaerobic microcosms experiments

Table 39: Results of the microcosm studies under different redox conditions, summarizing lag phase of transformation as well as formation and further degradation of metabolites formed.

compound	lag phase in days			formation of hydroxylated quinoline or isoquinoline compound			transformation of hydroxylated quinoline or isoquinoline compound		
	sulfate	iron (III)	nitrate	sulfate	iron (III)	nitrate	sulfate	iron (III)	nitrate
quinoline	+ (31)	+ (11)	+ (69)	+	+		+	+	
isoquinoline	+ (31)	- (>300)	+ (20)	+	(+)	+	+	-	-
2-methylquinoline	- (>300)	- (>300)	- (>300)						
3-methylquinoline	+ (31)	- (>300)	- (>300)	+			-		
4-methylquinoline	+ (31)	- (>300)	- (>300)	+			+		
6-methylquinoline	+ (20)	+ (153)	- (>300)	+	(+)	(+)	-	-	-
7-methylquinoline	+ (153)	- (>300)	- (>300)	+	(+)	(+)	-	-	-
8-methylquinoline	+ (20)	- (>300)	- (>300)	+	(+)	(+)	-	-	-
2,4-dimethylquinoline	- (>300)	- (>300)	- (>300)						
2,6-dimethylquinoline	- (>300)	- (>300)	- (>300)						
1(2H)-isoquinolinone	+ (213)	- (>300)	- (>300)						
2(1H)-quinolinone	+ (213)	+ (110)	+ (11)						
3,4-dihydro-2(1H)-quinolinone	+ (20)	+ (11)	+ (11)						
4-methyl-2(1H)-quinolinone	+ (213)	+ (69)	- (>300)						
2-methyl-4(1H)-quinolinone	- (>300)	- (>300)	- (>300)						
4(1H)-quinolinone	+ (153)	+ (69)	+ (110)	+	(+)	+	+	+	+
3(2H)-isoquinolinone	+ (213)	+ (110)	+ (110)		+	+		-	-
5-isoquinolinol	+ (300)	- (>300)	+ (300)	+			-		
7-isoquinolinol	+ (110)	- (>300)	- (>300)	+			-		

+ = >20 % transformation: the number in parentheses marks the time interval after which more than 20 % of the compound has been eliminated. Day 11 was the first day of sampling; - = no transformation within the time of experiments; (+) = only minor amounts of transformation product were detected.
Due to the heterogeneous composition of the soil as inoculum, parallel batches generally showed some variances concerning transformation rates as well as lag phases. For clear identification of transformation processes, the results with shortest lag phase and largest rate of transformation are given here.

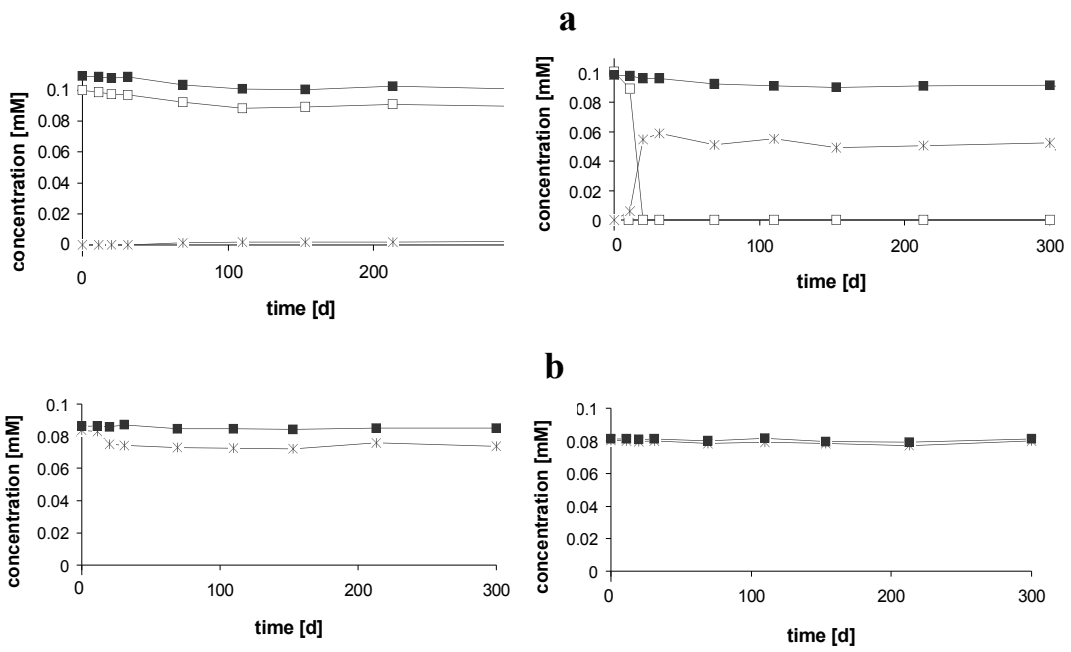


Figure 56: Transformation of isoquinoline (a) and 1(2H)-isoquinolinone (b) under iron- (left-hand side) and nitrate-reducing (right-hand side) conditions. Legend: isoquinoline (□), 1(2H)-isoquinolinone (⤫), abiotic control (■).

Table 40: Correlation of end acceptor used and mineralization of quinoline compound.

compound	- decrease of sulfate (mg/L) - decrease of compound (mg/L) - mineralization (%)	- decrease of nitrate (mg/L) - decrease of compound (mg/L) - mineralization (%)	- increase of iron (II) (mg/L) - decrease of compound (mg/L) - mineralization (%)
quinoline	31.1 9.7 84	72.4 10.7 177	28.3 10.9 15
2(1H)-quinolinone	24.9 10.8 73	35.1 12.3 88	13 11.8 8
3,4-dihydro-2(1H)-quinolinone	83 23 113	140.5 29.6 141	88 40.7 14
isoquinoline	12.2 9 134	30.6 6.3 127	- - -
1(2H)-isoquinolinone	3.2 7.2 72	11.8 11.5 26	- - -
4-methylquinoline	36 11.5 81	11.8 11.5 26	- - -
4-methyl-2(1H)-quinolinone	39.5 11.89 86	n.a.	11.5 13.5 6
4(1H)-quinolinone	15 13.6 36	57 12.3 143	16 3.7 30
3(2H)-isoquinolinone	- - -	8.3 12.3 21	- - -

The decrease of electron acceptor was correlated to abiotic control microcosm. Stoichiometry of mineralization of quinoline compounds under nitrate-, iron- and sulfate-reducing conditions was performed according to Table 38.
- = no decrease within the experimental phase; n.a. = not analysed; results of the most active microcosm are presented.
An analytical error of IC-measurements of sulfate and nitrate of about ~10 mg/L has to be considered. In none of the iron-reducing abiotic microcosm dissolved iron (II) was detectable.

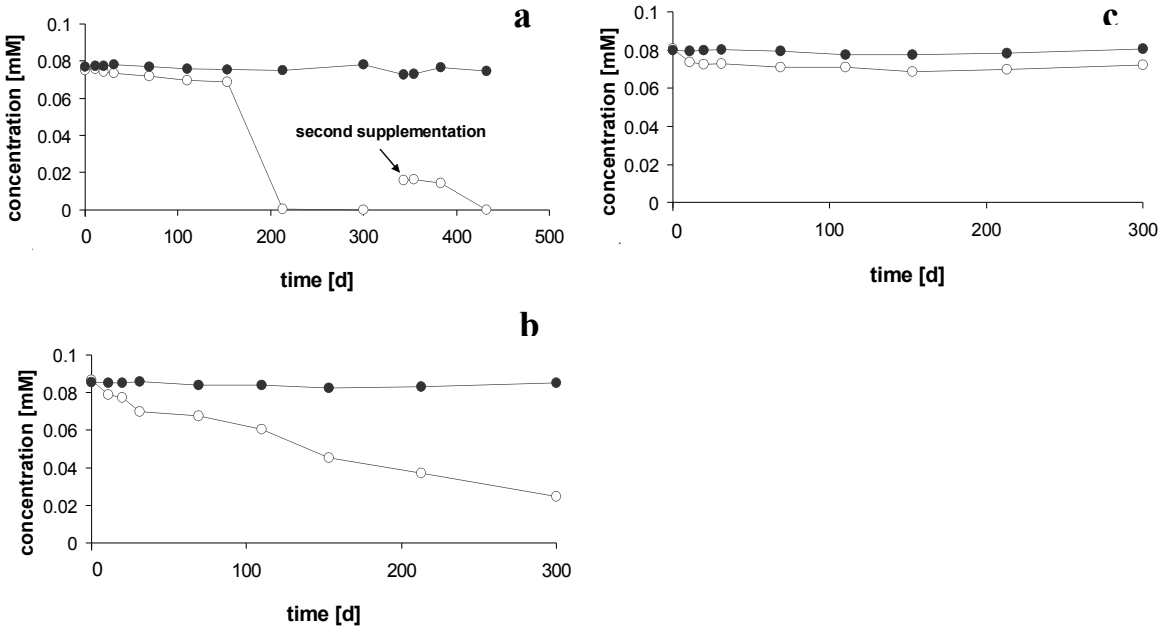


Figure 57: Degradation of 4-methyl-2(1H)-quinolinone under sulfate- (a), iron- (b) and nitrate-reducing (c) conditions. 4-methyl-2(1H)-quinolinone (○), abiotic control (●).

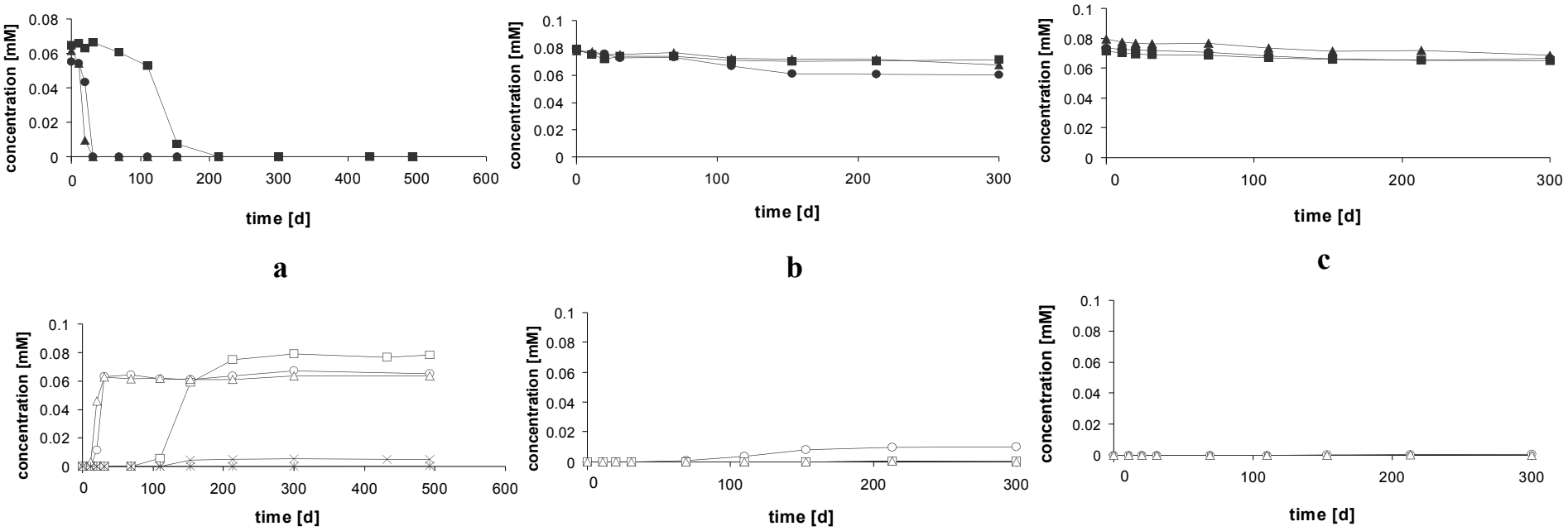


Figure 58: Degradation (upper row) of 6-, 7- and 8-methylquinoline and formation (lower row) of methyl-2(1H)-quinolinone metabolites under sulfate- (a), iron- (b) and nitrate-reducing (c) conditions. Legend: 6-methylquinoline (•), 7-methylquinoline (■), 8-methylquinoline (▲), 6-methyl-2(1H)-quinolinone (○), 7-methyl-2(1H)-quinolinone (□), 8-methyl-2(1H)-quinolinone (△), 6-methyl-3,4-dihydro-2(1H)-quinolinone (✳) and 7-methyl-3,4-dihydro-2(1H)-quinolinone (×).

Table 41: Results from the high-resolution measurements with Orbitrap for quinoline metabolites.

metabolite	empirical formula + H^+	measured mass +H^+ [g/mol]	mass difference to predicted mass [ppm]
2(1H)-quinolinone	$C_9H_8O_1N_1$	146.0600	-0.42
3,4-dihydro-2(1H)-quinolinone	$C_9H_{10}O_1N_1$	148.0757	0.33
3-methyl-2(1H)-quinolinone	$C_{10}H_{10}N_1O_1$	160.0753	-2.75
4-methyl-2(1H)-quinolinone	$C_{10}H_{10}N_1O_1$	160.0760	-1.25
4-methyl-3,4-dihydro-2(1H)-quinolinone	$C_{10}H_{12}N_1O_1$	162.0912	0.62
6-methyl-2(1H)-quinolinone	$C_{10}H_{10}N_1O_1$	160.0755	-1.38
6-methyl-3,4-dihydro-2(1H)-quinolinone	$C_{10}H_{12}N_1O_1$	162.0914	0.62
7-methyl-2(1H)-quinolinone	$C_{10}H_{10}N_1O_1$	160.0754	-2.00
7-methyl-3,4-dihydro-2(1H)-quinolinone	$C_{10}H_{12}N_1O_1$	162.0913	0.31
8-methyl-2(1H)-quinolinone	$C_{10}H_{10}N_1O_1$	160.0757	0.18
2,4-quinolinol	$C_9H_8O_2N_1$	162.0550	-0.03
1,5-isoquinolinol	$C_9H_8O_2N_1$	162.0549	-0.46
1,7-isoquinolinol	$C_9H_8O_2N_1$	162.0550	-0.22

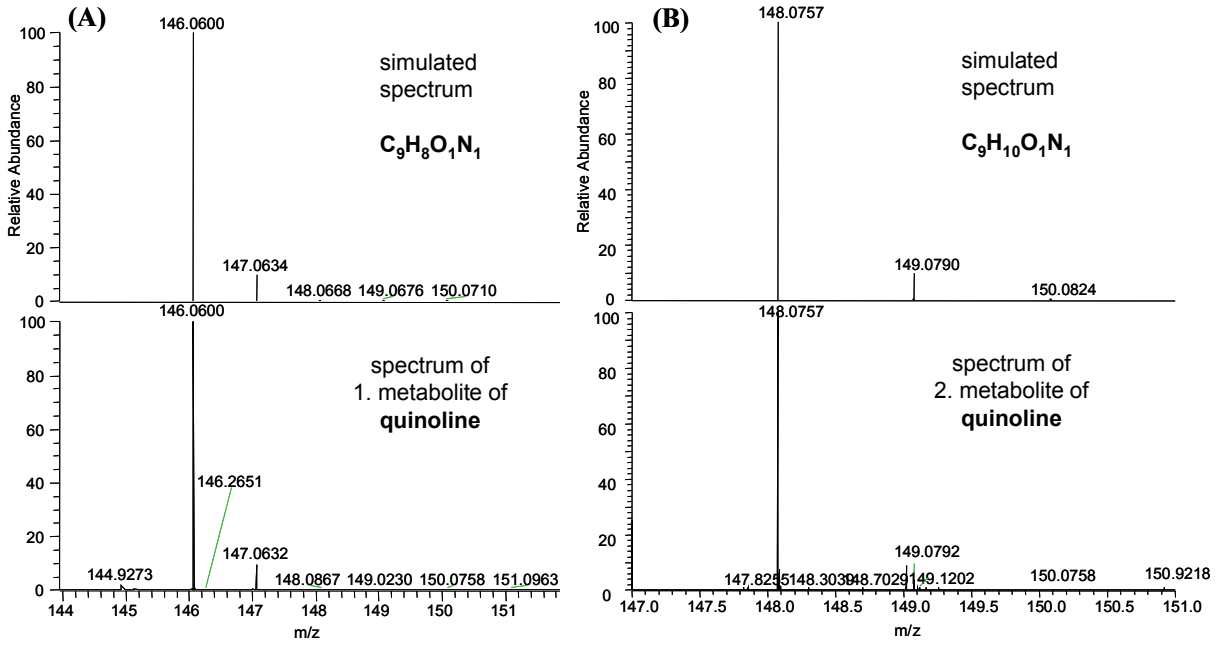


Figure 59: MS spectra of metabolites of quinoline: (A) 2(1H)-quinolinone and (B) 3,4-dihydro-2(1H)-quinolinone (bottom) as well as simulated MS spectra (top).

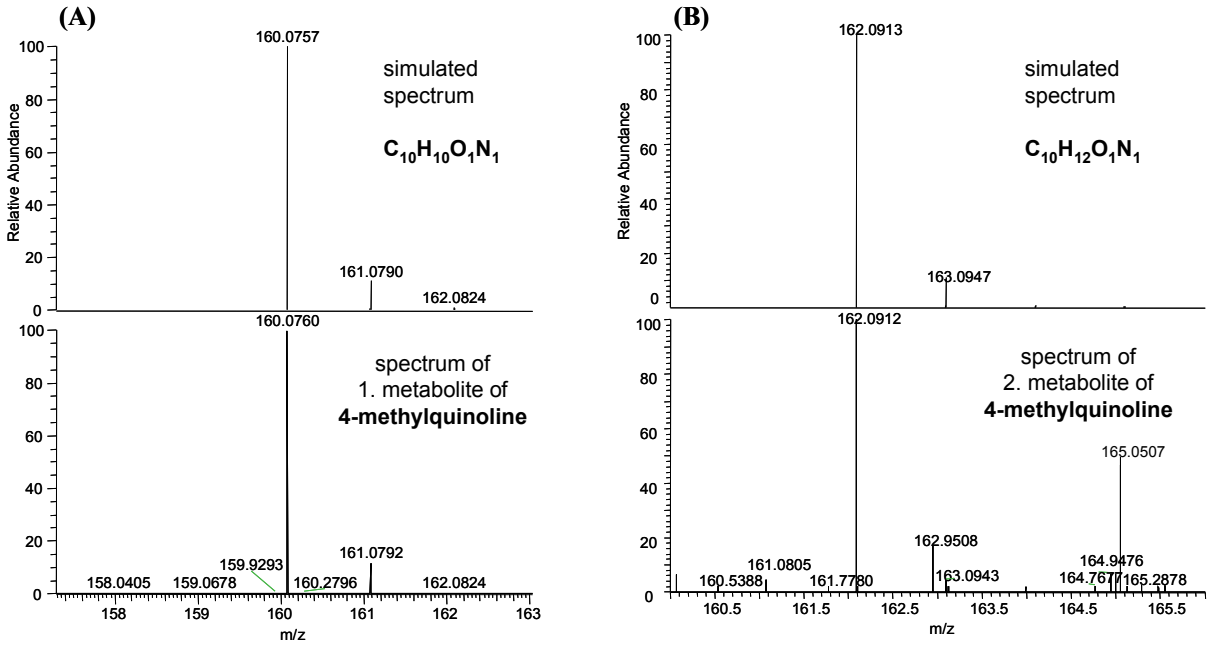


Figure 60: MS spectra of metabolites of 4-methylquinoline: (A) 4-methyl-2(1H)-quinolinone and (B) 4-methyl-3,4-dihydro-2(1H)-quinolinone (bottom) as well as simulated MS spectra (top).

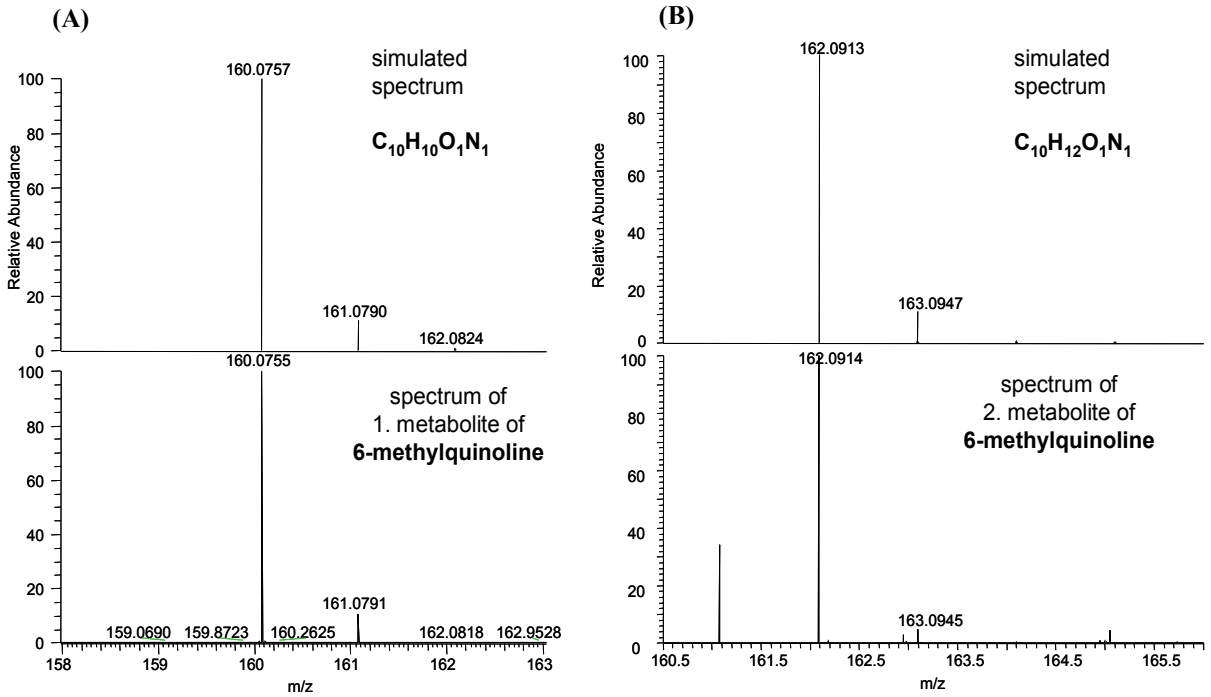


Figure 61: MS spectra of metabolites of 6-methylquinoline: (A) 6-methyl-2(1H)-quinolinone and (B) 6-methyl-3,4-dihydro-2(1H)-quinolinone (bottom) as well as simulated MS spectra (top).

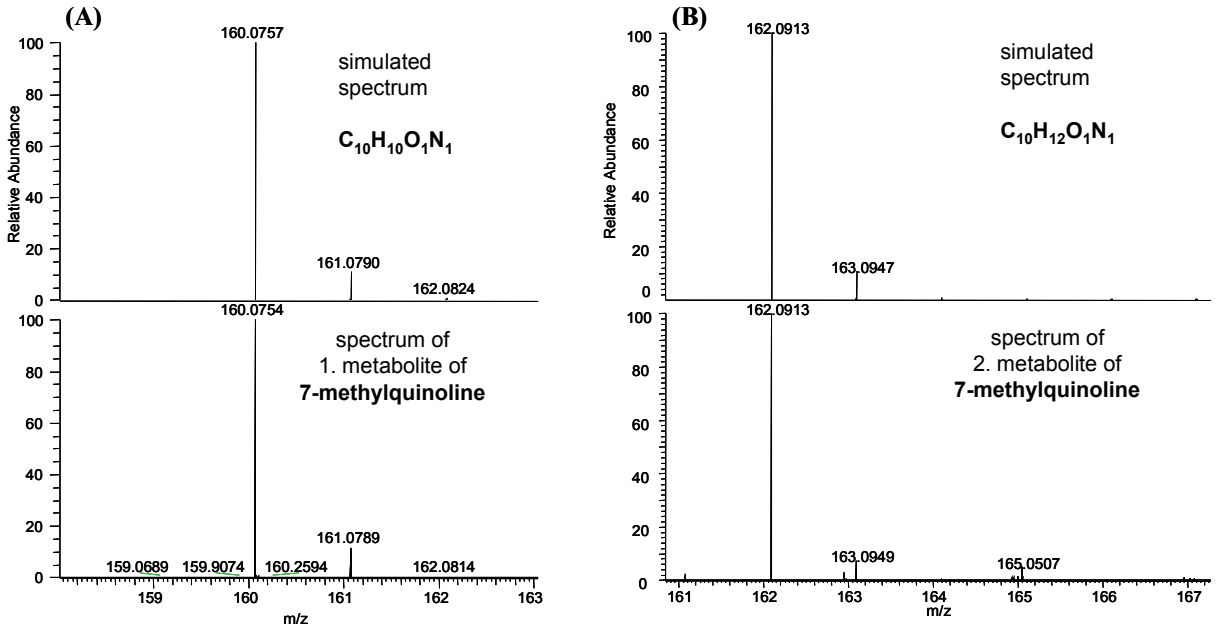


Figure 62: MS spectra of metabolites of 7-methylquinoline: (A) 7-methyl-2(1H)-quinolinone and (B) 7-methyl-3,4-dihydro-2(1H)-quinolinone (bottom) as well as simulated MS spectra (top).

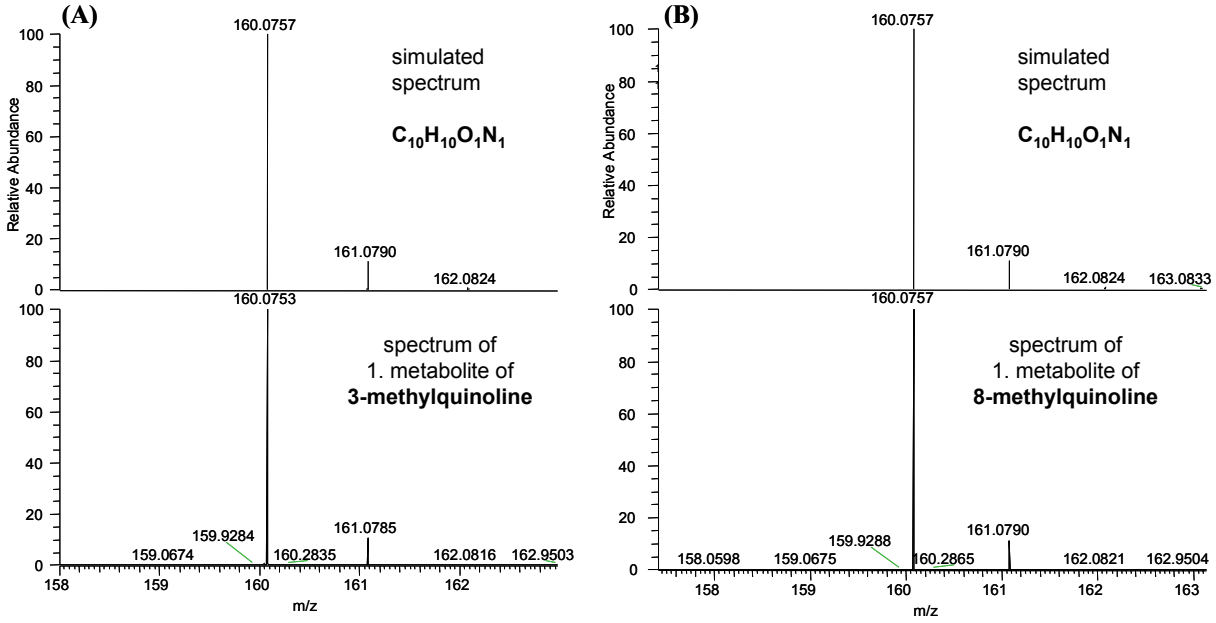


Figure 63: MS spectra of metabolites of 3-methylquinoline and 8-methylquinoline: (A) 3-methyl-2(1H)-quinolinone and (B) 8-methyl-2(1H)-quinolinone (bottom) as well as simulated MS spectra (top).

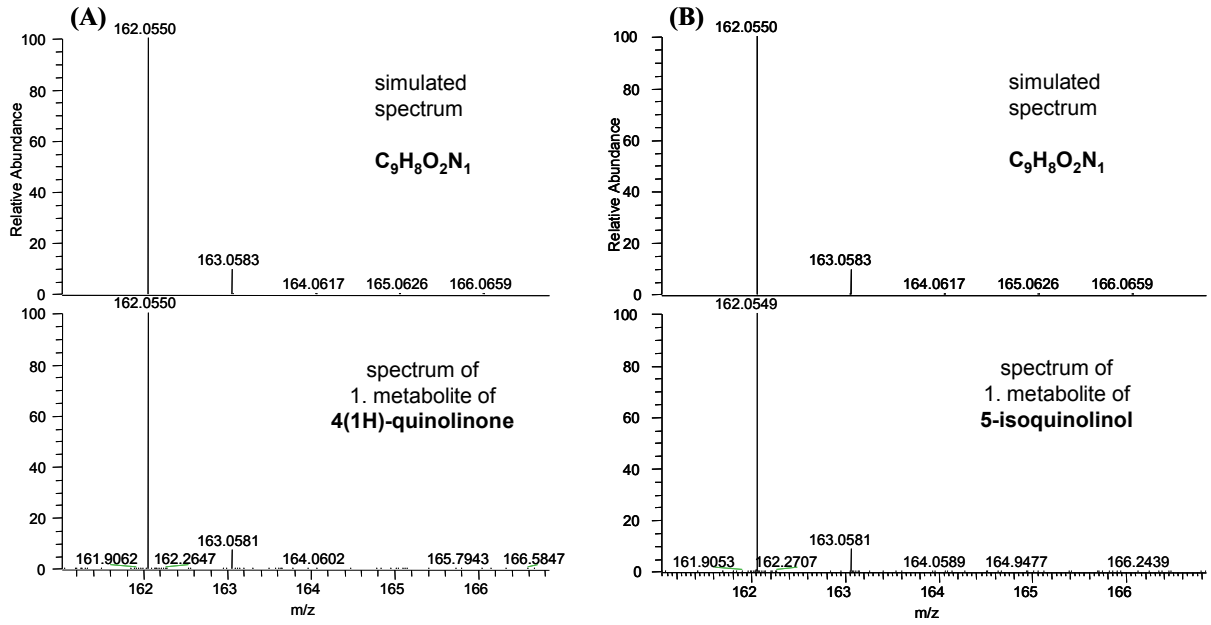


Figure 64: MS spectra of metabolites of 4(1H)-quinolinone and 5-isoquinolinol: (A) 2,4-quinolinol and (B) 1,5-isoquinolinol (bottom) as well as simulated MS spectra (top).

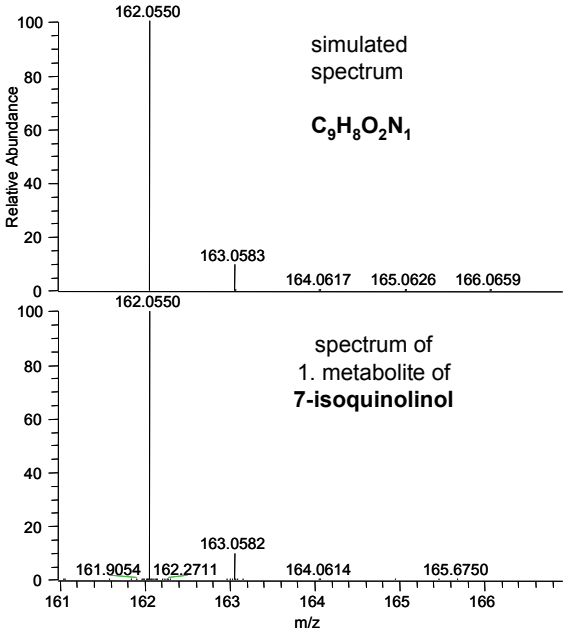


Figure 65: MS spectrum of first metabolite of 7-isoquinolinol: 1,7-isoquinolinol (bottom) and simulated MS spectrum (top).

7.6.3 Sorption of quinoline compounds

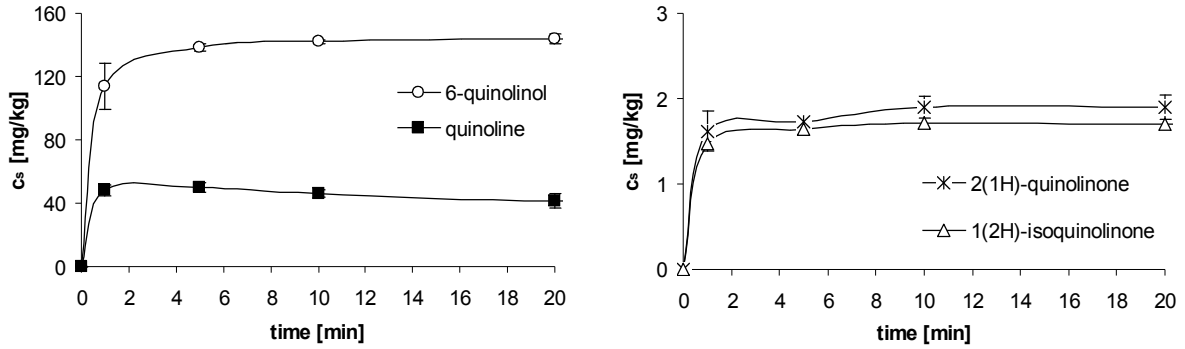


Figure 66: Sorption kinetics of quinoline and 6-quinolinol (left hand side) and 2(1H)-quinolinone and 1(2H)-isoquinolinone (right hand side) to montmorillonite.

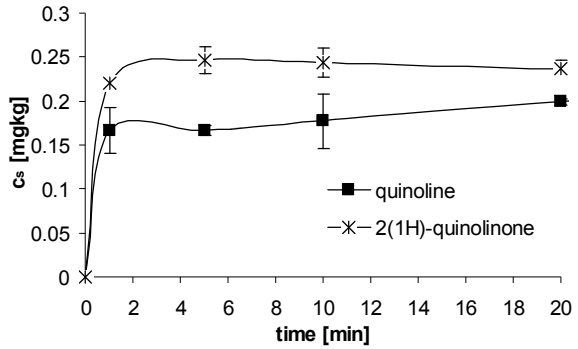


Figure 67: Sorption kinetics of quinoline and 2(1H)-quinolinone to aquifer material.

7.6.4 Hydroxylated quinolines as indication for natural attenuation

Table 42: Ratios metabolite/parent compound ($R_{metabolite}$) in groundwater samples of the testfield in Castrop-Rauxel.

$R_{metabolite}$	T1			T2			T3			T4			T5		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
$R_{2(1H)\text{-quinolinone}}$	0.8	1.1	0.2	0.2	n.a.	4.6	0.3	-	∞	0.2	2.3	-	0.2	0.3	∞
$R_{3,4\text{-dihydro-2(1H)-quinolinone}}$	x	x	x	x	n.a.	0.9	x	-	-	x	x	-	x	x	∞
$R_{1(2H)\text{-isoquinolinone}}$	3.1	4.9	33	1.5	n.a.	13	x	-	91.4	1.4	134	21	∞	3.8	136
$R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$	1.0	1.7	1.4	0.7	n.a.	0.5	0.5	∞	x	0.4	0.3	0.9	∞	x	x
$R_{4\text{-methyl-2(1H)-quinolinone}}$	0.9	0.6	0.2	x	n.a.	0.6	0.2	x	178	0.3	8.7	7.3	x	4.6	48
$\Sigma\ R_{methyl\text{-}2(1H)\text{-quinolinone}}$	31	56	14	20	n.a.	16	0.5	150	1530	6.6	280	477	4.2	82	52
$R_{metabolite}$	T6			T7			T8			T9			T10		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
$R_{2(1H)\text{-quinolinone}}$	6.9	4.0	8.6	6.5	8.2	67	28	115	57	8.6	1.4	111	0.4	0.3	1.0
$R_{3,4\text{-dihydro-2(1H)-quinolinone}}$	x	x	x	1.5	1.8	2.2	0.8	23	15	0.5	0.3	34	x	0.12	0.9
$R_{1(2H)\text{-isoquinolinone}}$	2.0	3.4	8.1	x	x	2.7	22	50	30	103	274	123	114	97	94
$R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$	x	x	x	0.1	<0.1	x	1.1	0.9	0.3	x	x	x	x	1.5	1.0
$R_{4\text{-methyl-2(1H)-quinolinone}}$	20	17	12	3.8	5.2	0.8	3.7	2.8	2.4	13	7.7	11	5.9	1.3	8.8
$\Sigma\ R_{methyl\text{-}2(1H)\text{-quinolinone}}$	24	31	37	20	31	7.5	11	13	12	30	73	29	58	8.4	73
$R_{metabolite}$	T11			T12			T13			T14			T15		
	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m	4 m	5 m	7 m
$R_{2(1H)\text{-quinolinone}}$	1.0	6.9	30	10	63	36	25	40	36	12	9.5	11	9.0	28	15
$R_{3,4\text{-dihydro-2(1H)-quinolinone}}$	x	1.4	4.0	3.0	3.5	0.4	6.4	9.8	6.9	2.8	3.7	4.3	2.8	8.1	6.2
$R_{1(2H)\text{-isoquinolinone}}$	1.2	5.4	6.2	15	22	23	32	34	18	20	37	30	137	599	154
$R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$	0.1	0.2	x	0.1	0.3	0.5	1.3	1.2	1.6	0.9	1.1	1.3	x	1.7	1.6
$R_{4\text{-methyl-2(1H)-quinolinone}}$	1.6	0.5	1.5	12	2.4	0.4	1.1	2.0	1.4	0.8	1.2	0.7	5.1	7.5	1.8
$\Sigma\ R_{methyl\text{-}2(1H)\text{-quinolinone}}$	<0.1	3.0	8.1	63	18	3.5	4.0	7.7	7.4	2.5	2.9	1.8	46	24	5.9

n.a. = not analysed; - = none of the both compounds detectable; ∞ = parent compound not detectable, x = only parent compound detectable.

Table 43: Ratios metabolite/parent compound ($R_{metabolite}$) of groundwater samples of the testfield in Düsseldorf-Flingern.

$R_{metabolite}$	19200				19201					19202					19203					19214				
	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10m	6.5 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m
$R_{2(1H)\text{-quinolinone}}$	∞	∞	∞	∞	36	3.2	1.5	1.2	1.0	21	7.6	1.3	0.94	6.1	19.5	11	1.2	1.1	-	32	6.4	x	0.88	1.1
$R_{3,4\text{-dihydro-2(1H)-quinolinone}}$	∞	∞	-	∞	14	0.23	1.8	1.0	0.76	3.7	1.2	x	x	2.1	2.2	2.3	0.80	0.40	-	2.7	0.80	x	0.25	0.64
$R_{1(2H)\text{-isoquinolinone}}$	696	5.6	2.8	2.0	16	13	23	2.9	0.86	166	28	3.7	1.7	2.0	28	60	3.6	2.2	1.8	51	26	2.2	2.4	1.5
$R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$	0.16	0.06	0.04	0.07	< 0.01	< 0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.04	0.04	0.01	0.01	0.02	0.02	0.04	0.02	0.03	0.04	0.04	0.07
$R_{4\text{-methyl-2(1H)-quinolinone}}$	292	2.4	70	3.7	94	12	2.3	43	2.3	116	6.4	3.1	3.1	43	16	12	3.5	7.4	∞	62	∞	x	1.7	0.97
$\Sigma\ R_{methyl\text{-2(1H)-quinolinone}}$	4.6	3.2	1.7	0.73	0.80	0.81	1.9	0.69	2.7	1.6	0.18	0.35	1.9	< 0.01	1.0	0.50	0.37	0.24	0.07	0.29	0.15	0.09	0.47	0.16

$R_{metabolite}$	19185			19213				19186					19206				19205				
	8 m	9 m	10 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m
$R_{2(1H)\text{-quinolinone}}$	∞	2.0	∞	∞	∞	0.40	1.2	0.88	-	0.57	-	x	∞	4.1	3.0	∞	∞	∞	∞	∞	0.52
$R_{3,4\text{-dihydro-2(1H)-quinolinone}}$	∞	0.76	∞	-	-	x	0.74	2.7	-	x	-	x	-	x	x	-	-	-	-	0.68	0.26
$R_{1(2H)\text{-isoquinolinone}}$	176	4.8	1.4	9.6	8.2	3.1	1.7	1.6	1.4	0.11	x	0.15	0.6	2.4	1.0	0.19	0.87	1.7	1.5	1.1	0.11
$R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$	0.01	0.02	0.03	0.04	0.08	0.03	0.04	0.08	0.05	0.07	0.05	0.06	0.07	0.06	0.03	0.03	0.05	0.04	0.04	0.03	0.06
$R_{4\text{-methyl-2(1H)-quinolinone}}$	107	3.5	8.1	1.3	2.2	1.5	2.6	5.8	2.0	0.73	-	x	3.0	4.1	142	-	3.2	x	12.7	3.0	x
$\Sigma\ R_{methyl\text{-2(1H)-quinolinone}}$	1.8	1.4	1.3	0.72	0.28	0.32	1.3	0.09	0.10	0.10	0.03	0.06	3.8	3.0	4.6	0.69	2.3	1.4	0.49	0.56	0.56

$R_{metabolite}$	19064			19209				19210					19071
	10 m	12 m	13 m	7 m	8 m	9 m	10 m	6.5 m	7 m	8 m	9 m	10 m	
$R_{2(1H)\text{-quinolinone}}$	-	-	-	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
$R_{3,4\text{-dihydro-2(1H)-quinolinone}}$	-	-	-	∞	∞	∞	∞	-	-	-	-	-	-
$R_{1(2H)\text{-isoquinolinone}}$	x	x	x	3.6	36	18	1.4	1.2	1.93	0.55	0.10	0.26	0.72
$R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$	0.05	0.05	0.04	0.14	0.14	0.03	0.04	0.08	0.06	0.04	0.04	0.04	0.17
$R_{4\text{-methyl-2(1H)-quinolinone}}$	-	-	-	1092	712	13	7.6	19	13.0	-	-	-	∞
$\Sigma\ R_{methyl\text{-2(1H)-quinolinone}}$	0.66	0.58	0.46	12	8.8	7.9	5.2	4.5	2.53	0.17	0.47	0.33	6.2

n.a. = not analysed; - = none of the both compounds detectable; ∞ = parent compound not detectable, x = only parent compound detectable

Table 44: Ratios metabolite/parent compound ($R_{metabolite}$) of groundwater samples of the testfield in Wülknitz.

$R_{metabolite}$	20/05			24/05			21/05	12/98		13/01		06/98		14/01	
	4-8 m	13-17 m	26-30 m	4-8 m	12-16 m	26-30 m	10-14 m	19-21 m	27-29 m	13-15 m	26-30 m	20-22 m	29-31 m	16-20 m	27- 31 m
$R_{2(1H)\text{-quinolinone}}$	2.9	3.9	3.1	∞	∞	2.9	4.7	∞	∞	∞	2.5	∞	x	∞	∞
$R_{3,4\text{-dihydro-2(1H)-quinolinone}}$	0.9	0.06	17	-	∞	2.5	0.4	-	-	∞	0.8	-	x	-	-
$R_{1(2H)\text{-isoquinolinone}}$	126	3.3	346	30	1188	175	5.1	∞	∞	135	290	x	∞	-	-
$R_{3,4\text{-dihydro-1(2H)-isoquinolinone}}$	1.3	0.15	25	1.6	1.3	3.4	0.04	∞	∞	3.5	1.7	3.1	∞	∞	∞
$R_{4\text{-methyl-2(1H)-quinolinone}}$	20	1.1	24	0.58	54	4.8	1.3	2.2	-	1.9	12	2.2	-	∞	∞
$\sum R_{methyl\text{-2(1H)-quinolinone}}$	45	22	1.4	21	83	58	2.4	16	4.5	52	1.8	24	1.2	31	3.2

n.a. = not analysed; - = none of the both compounds detectable; ∞ = parent compound not detectable, x = only parent compound detectable

Table 45: Homocycles and Heterocycles in several tar oil products. Concentration [mg/L].

Tar oil no.	1	2	3	4	5	6	7
N-Heterocycles							
quinoline	49	319	533	44	2753	3173	266
isoquinoline	29	143	326	21	985	1217	114
1-methylisoquinoline	5.1	13	303	6.4	52	79	53
3-methylisoquinoline	12	35	69	14	115	241	12
2-methylquinoline	33	104	232	33	565	1062	53
4-methylquinoline	30	163	346	29	387	1001	58
6-methylquinoline	22	69	141	14	175	496	27
Σ methylquinolines	95	406	779	260	2449	2378	143
2(1H)-quinolinone	151	267	241	118	44	1014	21
1(2H)-isoquinolinone	106	165	172	105	31	51	10
4-(1H)-quinolinone	0.7	0.6	1.0	8.1	3.1	0.2	0.6
5(1H)-quinolinone	0.8	1.0	6.3	3.6	0.9	1.8	1.0
6-quinolinol	1.4	1.0	2.5	4.0	0.6	0.2	1.6
7-isoquinolinol	5.1	4.8	9.7	6.8	2.1	0.3	0.8
3(2H)-isoquinolinone	<0.4	<0.4	<0.4	14	116	<0.4	<0.4
3,4-dihydro-2(1H)-quinolinone	44	46	67	49	2.4	10	17
3,4-dihydro-2(1H)-isoquinolinone	1.9	3.2	4.3	5.2	<0.6	0.1	0.6
2-methyl-6-quinolinol	1.1	1.2	4.5	3.7	1.1	<0.3	0.5
4-methyl-2(1H)-quinolinone	48	85	95	38	17	201	6.1
6-methoxyquinoline	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
1-methyl-2(1H)-quinolinone	<0.5	<0.5	30	<0.5	<0.5	<0.5	1.6
2-methyl-4(1H)-quinolinone	0.2	1.2	2.3	<0.6	0.8	<0.6	<0.6
Σ methyl-2(1H)-quinolinones	176	357	466	201	79	514	25
2,4-dimethylquinoline	26	101	188	24	151	527	30
2,6-dimethylquinoline	6.9	27	54	8.4	49	201	9.1
1,2,3,4-tetrahydroquinoline	<1.3	<1.3	<1.3	<1.3	<1.3	7.3	<1.3
7-hydroxy-4-methyl-2(1H)-quinolinone	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
4-hydroxy-1-methyl-2(1H)-quinolinone	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6
carbazole	1179	2433	9388	5120	10807	8977	4905
acridine	4775	5658	13608	6295	3024	<240	955
9(10H)-acridinone	<240	616	<240	<240	<240	512	365
6(5H)-phenanthridinone	<40	1479	384	<40	2473	5276	59
O-Heterocycles							
benzofuran	<40	<40	<40	<40	223.	675	<40
2-methylbenzofuran	<40	<40	<40	<40	<40	1881	<40
dibenzofuran	14291	17256	2716	17649	21469	32496	4629
2,3-dimethylbenzofuran	<40	<40	<40	<40	<40	<40	<40
S-Heterocycles							
benzothiophene	<80	<80	<80	<80	5157	6375	274
dibenzothiophene	<80	<80	<80	<80	<80	<80	<80
PAHs/Homocycles							
indene	<40	<40	<40	<40	<40	4838	<40
naphthalene	11487	10891	1285	5667	63835	58917	5588
indan	<160	<160	<160	<160	<160	8857	<160
1-methylnaphthalene	54203	28205	14818	9747	7416	18569	867
2-methylnaphthalene	59145	34693	20392	30621	27812	42966	1922
fluorene	45913	29998	36692	57539	31722	40470	6314
acenaphthene	57819	51185	5028	37851	1199	47681	3972
1-indanone	386	163	<40	<40	<40	160	<40
1-acenaphthenol	505	52	<20	<20	<20	<20	<20
2-naphthol	1274	321	960	787	<40	<40	<40
1-naphthol	749	<40	1034	243	410	<40	125
1,3-dimethylnaphthalene	<40	<40	<40	<40	<40	24424	<40

7.7 List of publications

Reineke, A.-K., Göen, T., Preiß, A., Hollender, J. 2007. Quinoline and derivatives at a tar oil contaminated site: hydroxylated products as indicator for natural attenuation? *Environ. Sci. Technol.* **41**: 5314-5322.

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Blotevogel, J., Reineke, A.-K., Hollender, J., Held, T. 2006. Überwachung von NSO-Heterocyclen an Teeröl-kontaminierten Standorten. *Grundwasser-Zeitschrift der Fachsektion Hydrogeologie* **11**: 295-297.

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Reineke, A.-K., Blotevogel, J., Held, T., Hollender, J. 2005. Heterocyclische Aromaten und andere teeröltypische Schadstoffe im Grundwasser – Vorkommen, Abbaupotential und Bewertung: In: Tagungsband 2. BMBF Statusseminar KORA in Frankfurt a.M., S. 73-81, ISBN 3-89746-071-8.

Hollender, J., Mundt M., Reineke A., Rippen, G., Held, T. 2004. Heterocyclische Aromaten und andere teeröltypische Schadstoffe im Grundwasser – Vorkommen, Abbaupotential und Bewertung: In: Tagungsband 1. BMBF Statusseminar KORA in Leipzig, S. 54-61.

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Reineke, A.-K., J. Hollender. 2005. Vorkommen und Verbleib von NSO-heterocyclischen Verbindungen im Grundwasser belasteter Standorte, Stand Juni 2005. KORA-TV2 - Statusgespräch in Düsseldorf.

Reineke, A.-K., M. Mundt, J. Hollender. 2004. Vorkommen und Verbleib von NSO-heterocyclischen Verbindungen im Grundwasser belasteter Standorte. 2004. KORA-TV2 - Statusgespräch in Castrop-Rauxel.

Poster presentations

Reineke, A.-K., W. Dott, J. Hollender. 2007. Hydroxylated products of quinoline as indicator for natural attenuation? Micropol and Ecohazard, Frankfurt.

Reineke, A.-K., T. Göen, A. Preiß, J. Hollender. 2006. Microbial degradation of quinoline and its derivatives at tar oil contaminated sites. SETAC Europe 2006 in The Hague, The Netherlands, book of abstracts, p. 150.

Reineke, A.-K., Mundt, M., Hollender J. 2005. Tar oil compounds in groundwater: Monitoring of NSO-heterocyclic compounds and determination of the microbial degradation potential. ConSoil 2005 , 3.-7. Oktober in Bordeaux, France. CD of abstracts, p. 2795-2803.

Reineke, A.-K., Mundt, M., Hollender, J. 2005. Monitoring von NSO-heterocyclischen und homocyclischen Teerölverbindungen: Vorkommen und Verbleib an belasteten Standorten. In: Proceedingband GDCh-Tagung der Wasserchemischen Gesellschaft in Bad Mergentheim, ISBN 3-936028-29-X, S. 436-438.

Blotevogel, J., Held, T., Reineke, A., Hollender, J. 2005. Heterocyclische Aromaten und andere teeröltypische Schadstoffe im Grundwasser: Identifikation von Prioritärsubstanzen anhand von Stoffeigenschaften und Vorkommen an kontaminierten Standorten. 2. BMBF Statusseminar KORA in Frankfurt a.M.

Reineke, A.-K., Hollender J. 2004. Heterocyclische Aromaten und andere teeröltypische Schadstoffe im Grundwasser: Identifizierung, Quantifizierung und Untersuchung der Metabolisierung. 1. BMBF Statusseminar KORA in Leipzig.

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01/2002 – 03/2002	Projektarbeit im Pflanzenschutzzentrum der Bayer-AG in Monheim (Institut für Metabolismusforschung und Rückstandsanalytik)

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04/2006 – 09/2006	Aufenthalt in der Schweiz, Anstellung an der Eidgenössischen Anstalt für Wasser und Abwasser (Eawag) in Dübendorf, Abteilung Umweltchemie
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