



The dissolution and microbial degradation of mobile aromatic hydrocarbons from a Pintsch gas tar DNAPL source zone

J.A. Van Leeuwen^{a,e,*}, N. Hartog^{a,b}, J. Gerritse^e, C. Gallacher^c, R. Helmus^d, O. Brock^d, J.R. Parsons^d, S.M. Hassanizadeh^a

^a Utrecht University, Princetonplein 9, Utrecht 3584 CC, Netherlands

^b KWR Water Cycle Research Institute, Groningenhaven 7, Nieuwegein 3433 PE, Netherlands

^c University of Strathclyde, 75 Montrose St., Glasgow, UK

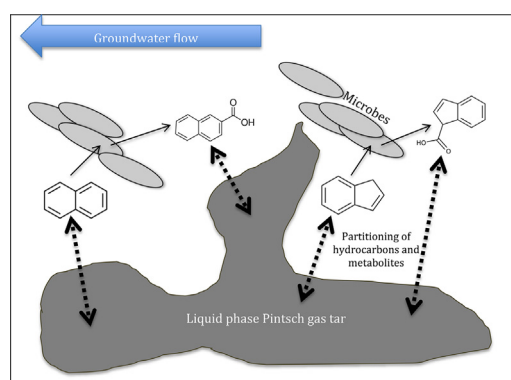
^d University of Amsterdam, IBED, Science Park 904, Amsterdam 1098 XH, Netherlands

^e Deltares, Princetonlaan 8, Utrecht 3584 CB, Netherlands

HIGHLIGHTS

- Phenanthrene is the primary component in Pintsch gas tar, rather than naphthalene.
- 47 metabolites from anaerobic biodegradation of aromatic hydrocarbons were found.
- Detected metabolites suggest active anaerobic biodegradation at pool zone conditions.
- A novel biodegradation pathway is proposed, based on 11 novel indene metabolites.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 24 September 2019

Received in revised form 3 March 2020

Accepted 6 March 2020

Available online 8 March 2020

Keywords:

Pintsch gas tar

Tar composition

DNAPL

Anaerobic degradation indene

Metabolite profiling

Partitioning

ABSTRACT

Source zones containing tar, a dense non-aqueous phase liquid (DNAPL), can contaminate groundwater for centuries. A common occurrence of tar is at former Pintsch gas factories. Little is known about the composition and fate of contaminants dissolving from Pintsch gas tar DNAPL. In this study, we determined the composition and water-soluble characteristics of mobile aromatic hydrocarbons and their biodegradation metabolites in the DNAPL contaminated groundwater at a former Pintsch gas tar plant. We assessed the factors that determine the fate of observed groundwater contaminants. Measured values of density ($1.03\text{--}1.06\text{ kg/m}^3$) and viscosity ($18.6\text{--}39.4\text{ cP}$) were found to be relatively low compared to common coal tars. Analysis showed that unlike common coal tars phenanthrene is the primary component rather than naphthalene. Moreover, it was found that Pintsch gas tar contains a relatively high amount of light molecular aromatic hydrocarbon compounds, such as benzene, toluene, ethylbenzene and xylenes (BTEX). Less commonly reported components, such as styrene, ethyltoluenes, di-ethylbenzene, 1,2,4,5-tetramethylbenzene, were also detected in water extracts from Pintsch gas tar. Moreover, 46 relatively hydrophilic metabolites were found within the tar samples. Metabolites present within the tar suggest biodegradation of mobile aromatic Pintsch gas tar compounds occurred near the DNAPL. Based on eleven detected suspect metabolites, a novel anaerobic biodegradation pathway is proposed for indene. Overall, our findings indicate that Pintsch gas tar has higher invasive and higher flux properties than most coal

* Corresponding author at: Utrecht University, Princetonplein 9, Utrecht 3584 CC, Netherlands.
E-mail address: j.a.vanleeuwen@uu.nl (J.A. Van Leeuwen).

tars due to its relatively low density, low viscosity and, high content of water-soluble compounds. The partitioning of contaminants from multi-component DNAPL into the aqueous phase and re-dissolution of their slightly less hydrophobic metabolites back from the aqueous phase into the DNAPL is feasible and demonstrates the complexity of assessing degradation processes within a source zone.

Crown Copyright © 2020 Published by Elsevier B.V. All rights reserved.

1. Introduction

Former manufactured gas plants (MGPs) are major sources of groundwater contamination (Birak and Miller, 2009). In Europe, around 4500 former MGPs exist (Thomas and Brinckerhoff, 2014) and in the US the estimated number of former MGP sites ranges from 1000 to 2650 (Hatheway and Group, 2012a). Pintsch gas is a specific type of oil gas that was used extensively to light railway coaches (American Scientific, 1898). Liquid phase tar is a waste product formed during the gasification process at MGP sites. Its presence in the subsurface is usually due to leakage from pipes, tanks, and storage chambers, or deliberate dumping (Thomas and Churchill, 2014). Once in the subsurface, both residual- and pool zone tar can act as a groundwater contamination source for decades or even longer (Kueper et al., 2003). Tars are complex mixtures consisting of hundreds of hydrocarbon compounds (Peters and Luthy, 1993). The composition of tars is, in general, highly variable due to differences in especially the type of feedstock, but also gasification processes, retort oven design and the processing temperature (Gallacher et al., 2017a; Johnston et al., 1993; McGregor et al., 2012; McRae et al., 1999). Our knowledge of the composition of tars is rather limited (Bruce et al., 1991; Electric Power Research Institute, 1993). With increasing production temperature, higher percentages of benzene and toluene are formed (Egloff and Twomey, 1916). An estimated 5 to 10% of all MGP were Pintsch gas plants, mostly situated at railway sites (Hatheway and Group, 2012b). However, very little research into this type of contamination has been done (Wanior and Ripper, 1993).

The aim of our study was to gain a better understanding of Pintsch gas tar and its potential environmental impact on the saturated subsurface. Therefore, we performed a detailed site investigation at a former Pintsch gas tar factory to determine the composition and physical properties of the Pintsch gas tar as dense non-aqueous phase liquid (DNAPL) from a contaminated site in detail in order to determine its impact on groundwater quality, and the potential for degradation. From multiple tar samples, we investigated the partitioning behaviour of the water-

soluble hydrocarbons leaching from the tar into groundwater and dilution effects, potentially caused by heterogeneity in tar distribution. Moreover, we looked into hydrocarbon compounds produced from biological activity at pooled tar zone conditions. Finally, we determined the water-soluble components of the tar by partitioning experiments on liquid phase tar samples and analysed potential metabolites formed by anaerobic biodegradation. The overall aim of the study was to gain a better understanding of the risk posed by sites contaminated by Pintsch gas tar.

2. Materials and methods

2.1. Site description

The field site in this study is a former MGP located adjacent to the central railway station in the city of Amersfoort, the Netherlands (Fig. 1). From 1910 to 1958, the factory produced lighting gas via the Pintsch gas process, and the tar by-product was dumped onsite in unlined waste lagoons. The site is situated at a geological transition area, where horizontal sand, peat on loam, and gravel layers shift to a more vertical, glacial pushed moraine formation to the south. The aquitard at 12 meter below ground surface (mbgs) consists of a peat on loam layer, thinning from north to south. At the western domain of the source zone area, the aquitard is <50 cm thick, probably resulting in loss of integrity of the aquitard. The source zone is considered to be the pool zone and residual zone combined. In the pool zone, saturation of tar is high enough for the DNAPL to potentially flow as a separate phase. In the residual zone, saturation of the tar is relatively low, and the liquid tar is entrapped, and therefore not considered mobile. The source zone area covers $\pm 4500 \text{ m}^2$, containing the pooled and residual tar, whose volumes are estimated at 2.8 million liters and 250,000 L, respectively. Residual tar is present below the former tar lagoon, with a soil volume of approximately $16,500 \text{ m}^3$, which extends above the pooled tar zone (Figs. 1 and 2). The pooled tar zone is approximately $11,000 \text{ m}^3$ of soil,

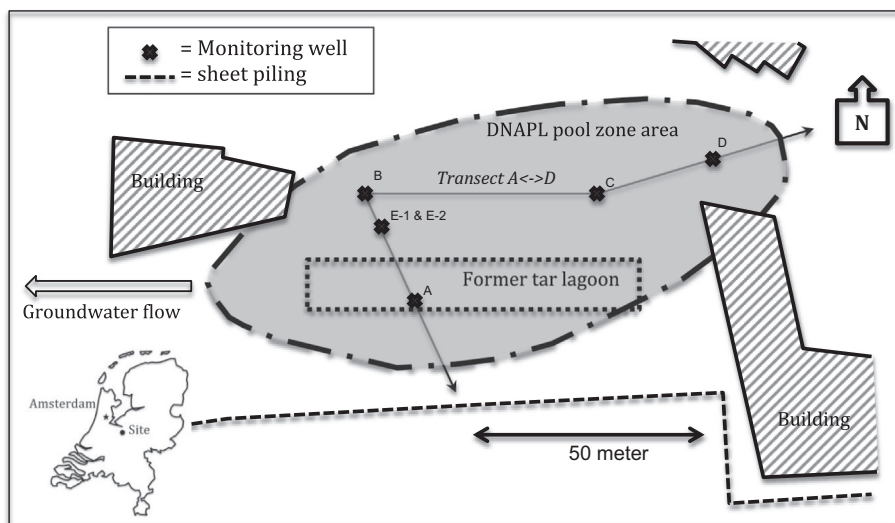


Fig. 1. DNAPL pool zone area visualizing monitoring well locations for sampling. The DNAPL source zone covers an area of approximately 4500 m^2 and visualized by the grey area.

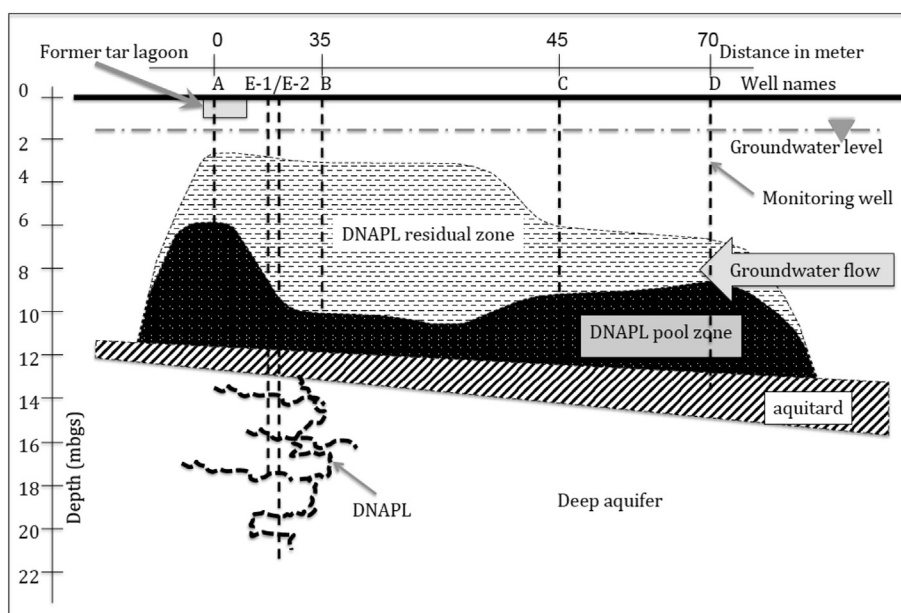


Fig. 2. Transect A→D source zone is considered the pooled Pintsch gas tar (black area) plus the DNAPL residual zone (striped area) above the pooled Pintsch gas tar, residual and pool height can be interpreted from meter below ground surface (mbgs).

located at the bottom of the shallow aquifer. There the tar has spread in the north-eastward dipping direction of the underlying thin aquitard.

In 2000, an engineered hydrogeological barrier was installed, using two vertical, steel sheet pile walls. The hydrogeological barrier forms a funnel-and-gate system in the shallow unconfined aquifer (Fig. 1). Its main purpose is containment of the source and pool zone area and treatment of the groundwater plume by aeration. The direction of groundwater flow in the shallow aquifer is towards the southeast and shifts at the hydrogeological barrier towards the south-west. As a result, the groundwater interacts with the upgradient DNAPL. The bulk of the DNAPL is situated below the former tar lagoon and is regarded as the point of entry in the subsurface and recognized as the source of the pool zone (Fig. 2). In most monitoring wells within the pool zone, the measured tar heights varied from 0.3 to 6 m from the bottom of the well. An outline schematic of the pool zone is given in Fig. 1.

2.2. Field work

Prior to our research, 40 boreholes had delineated the DNAPL pool zone. The wells were placed on the clay/peat aquitard. Pool height was monitored 20 times over a 19-month period. DNAPL monitoring well A is situated directly under the former tar lagoon, where the bulk of the liquid tar is present and the subsurface is saturated with tar from 6 to 12 mbgs. Tar from this well is considered to be the least weathered from site, and therefore reflect the most original composition. Weathering is considered to be relatively low at this well, due to the high pore saturation of tar and therefore relatively low tar to groundwater contact. The other three wells chosen for sampling represent the gravity-driven migrated liquid tar along the dipping aquitard towards the north. To gather tar for experimental use from the deep aquifer, two monitoring wells E-1 and E-2 were selected (17 and 21 mbgs) in the deep aquifer. Reference well pb323 (not shown in Fig. 1) was situated 140-meter upgradient from the source zone. Groundwater was collected from the reference well to determine the background composition of contaminants and metabolites and for use in the partitioning experiments. DNAPL levels in monitoring wells were measured using a Solinst interface probe model122. The liquid phase tar was sampled using a peristaltic Watson-Marlow 604 U/R pump, using low-density polyethylene (LDPE) tubing from the bottom of monitoring

wells at a flow rate of ± 1 L/h. At this rate, 10 to 60 L liquid phase tar was obtained per sampling well without extracting any groundwater.

Four Pintsch gas tar samples from four monitoring wells within the DNAPL pool zone were sent to SGS laboratories in Rotterdam, The Netherlands, to analyse tar viscosity, density and water content (Table 1). Analyses were performed according to ASTM D7042, D4052 and D1744 standards, respectively.

2.3. Pintsch gas tar composition

One liquid phase tar sample, extracted from well A, was analysed by comprehensive two-dimensional gas chromatography combined with fast time of flight mass spectrometry (GCxGC-TOFMS). A LECO (St. Joseph, Michigan) time of flight mass spectrometer on a model Pegasus 4D was connected to an Agilent 7890A gas chromatograph equipped with a LECO thermal modulator. The TOF ion source was fixed at 200 °C and masses between 45 and 500 μm were scanned at a 200 spectra/second rate. The detector voltage was set at 1700 V and the applied electron ionisation voltage at 70 eV.

The sample was prepared by accelerated solvent extraction (ASE) as described by Gallacher et al. (2017b). The sample was sealed and stored at 4 °C prior to analysis. Dichloromethane, used as a solvent for extraction of the Pintsch gas tar sample, was purchased from Fisher Scientific (Loughborough, U.K.). D₁₀-Phenanthrene from Sigma-Aldrich (Gillingham, U.K.) was purchased as a calibration standard. Calibration standards were prepared using a standard mixture containing all 16 EPA PAHs, purchased from Thames Restek (Saunderton Bucks, U.K.). Four deuterated PAHs were purchased from Sigma-Aldrich (Gillingham, U.K.). Extraction from the tar sample was performed using an ASE 350 Accelerated Solvent Extraction system (Dionex, Camberley, UK) and

Table 1

Physical properties for Pintsch gas tar samples, collected from wells A, B, C, D. No replicate measurements were conducted on these samples.

Well sample	A	B	C	D
Distance from entry point (m)	0	25	45	70
Viscosity (cP)	18.56	31.58	34.52	39.41
Density (kg/L)	1.0308	1.0498	1.0576	1.0593
Water content (% wt)	0.0800	0.0660	0.0580	0.0650

10-mL stainless steel extraction cells. A comprehensive description of the sample preparation prior to analysis is given in the Supplementary information.

All standards and extracts were analysed with the primary oven temperature programmed at 75 °C for 1 min, increasing by 12 °C/min to 120 °C, then increasing by 3 °C/min to 310 °C, and held at this temperature for 10 min. The secondary oven and modulator temperatures were programmed at 20 °C higher offset relative to the primary oven. The modulation period was 6 s with a 1.3 second hot pulse time and a cool time of 1.7 s. The injection port temperature was 250 °C, with a split ratio of 50. One microliter of sample was injected for each run using an MPS2 twister auto-sampler (Gerstel). Helium was used as the carrier gas, with a flow rate of 1.0 mL/min.

The primary column was a mid-polar TR-50 MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness, Thermo Scientific, Loughborough) and a secondary column, a). A non-polar Rtx-5SiIMS column (1.5 m × 0.25 mm i.d. × 0.25 µm film thickness) was used as a secondary column, supplied by Thames Restek. The columns were connected via a Thames Restek Press-tight connector.

The chromatograms were processed using LECO ChromaTOF software (Version 4.50.8.0) to search for and identify all peaks with a signal-to-noise (S/N) ratio >10 according to (Gallacher et al., 2017b), which is the limit of quantification. Signal to noise was set at this ratio to avoid mass phantom peaks. A signal-to-noise (S/N) ratio >10 is the limit of quantification. This signal to noise ratio was chosen to avoid mass phantom peaks, which could potentially hide other peaks for identification. Statistical analysis was carried out using Microsoft Excel (Version 14.3.7), Minitab version 16 and Matlab R2013a. All peak areas were normalised by dividing the peak area by the sample weight and the D₁₀-Phenanthrene peak area and taking the fourth root of the normalised value. Fourth root data formation is required as, without data pre-processing, the large range of peak intensities within the dataset results in small peaks contributing less towards the principal components regardless of their chemical importance. Taking the fourth root also allows the analysis to focus on the presence/absence of contaminants rather than their concentration, and so relates the data to the primary production method used, instead of changes in relative concentration, which may have occurred due to environmental factors.

2.4. Bench scale partitioning tests

To study the potential environmental impact of Pintsch gas tar on groundwater, two partitioning tests were performed. The first test was conducted to study the differences in partitioning behaviour of Pintsch gas tar samples taken from different locations in the pool zone. Maximum saturation for water soluble tar compounds was conducted using the method described by Lee et al. (1992a). This method resulted in a 1:4 tar to water ratio in the experiment.

The second partitioning test was conducted to determine the effects of increasing dilution effects for ratios on the partitioning of tar components. Differences in dilution in-situ are due to, for example, heterogeneity in the distribution of tar in the pool zone. Accordingly, tar was exposed to increasing water volumes and the water-soluble fraction of hydrocarbons was quantified. Approximately 2 mL of Pintsch gas tar, 12 mL of unimpacted groundwater from a shallow aquifer, were sampled at a location 175 meter upgradient and 2 mL of 0.5 g/L mercury chloride (HgCl₂) were added to a 20-mL glass vial with 4 mL of headspace and sealed with a Grace silicone, PTFE lined magnetic gold cap. The unimpacted groundwater contained 24.1 mg/L of nitrate, 35.0 mg/L of sulphate, and 0.1 mg/L of manganese (III). No benzene, toluene, ethylbenzene, xylenes (BTEX) nor naphthalene above the detection limit of <10 µg/L were identified. To produce equilibrium conditions for the dissolving tar components, the vials were rotated top over bottom on a Labinco 528 test tube rotator at 9 rpm at 12 °C for 4 weeks. Then, the samples were placed upside down in a vial rack and kept for 7 days in the dark at 12 °C according to the procedure

proposed by (Peters and Luthy, 1993). After equilibration, 2 × 1.5 mL of the aqueous phase was sampled and placed in clean 2-mL glass vials for further gas and liquid chromatography analysis. From the 2 mL vials, 100 µL subsamples were diluted in 12 mL demineralized water in 20 mL vials capped with PTFE-lined silicone septa for GC-MS headspace analysis.

Concentrations for BTEX partitioned into the water phase were used to determine the content originating from the tar sample for each of the detected monocyclic aromatic hydrocarbon (MAH) components by calculating partitioning coefficient for each compound.

The initial concentration (V_t) of mono-aromatic hydrocarbons (MAHs) in the tar and their partitioning coefficients were calculated from the partitioned concentrations in the aqueous phase following the methods outlined by Rixey (1999). The method assumes linear partitioning, where the equilibrium concentration of component i in the tar C_t^i and its aqueous concentrations C_{aq}^i can be related by their partitioning coefficient K_{tw}^i :

$$K_{tw}^i = \frac{C_t^i}{C_{aq}^i} \quad (1)$$

Also, a mass balance of component i gives:

$$V_t (C_t^{i,0} - C_t^i) = V_{aq} C_{aq}^i \quad (2)$$

where V_t is the volume of the tar, $C_t^{i,0}$ is the initial concentration in the tar, C_t^i is the concentration in the tar following the dissolution in the aqueous phase, V_{aq} is the water volume in the test bottle. C_{aq}^i is the aqueous concentration of component i . Combining Eqs. (1) and (2) gives:

$$\frac{1}{C_{aq}^i} = \frac{1}{C_t^{i,0}} \left(\frac{V_{aq}}{V_t} \right) + \frac{K_{tw}^i}{C_t^{i,0}} \quad (3)$$

The experiment is repeated with different volumes of water in different tests. Then, if a straight line can be fitted to the plot of $1/C_{aq}^i$ versus V_{aq}/V_t , from the slope, $1/C_t^{i,0}$, and the y-intercept, $K_{tw}^i/C_t^{i,0}$; K_{tw}^i and $C_t^{i,0}$ can be determined.

To quantify the most soluble tar components (MSTC), six treatments were prepared in duplicate. Also, two 1 L bottles in duplicate were prepared as control samples, where one sample contained only groundwater and no tar, and the second one was filled with demineralized water and tar. Groundwater mixtures were prepared with the tar with tar and groundwater from site. Tar from well A was used, as it is the least weathered and therefore considered to reflect the most unaltered tar obtainable from the site. Groundwater from reference well pb323 was used. The six treatments consisted of increasing tar to water ratios (10:1, 21:1, 43:1, 360:1, 722:1 and 3613:1) in batch bottles ranging from 100 mL to 10 L. Two control treatments were prepared, the first with only groundwater from site (no tar) and second with tar from site with demineralized water.

HgCl₂ was added at 25 mg/L in each treatment to inhibit any biodegradation. The bottles were sealed with butyl rubber stoppers and stirred with a magnetic bean at 150 rpm for five weeks in a dark room at a constant temperature of 12 °C. Subsequently, stirring was stopped and the bottles were kept at the same conditions for seven days to equilibrate. To prevent tar contamination during later sampling procedure, 150 mm long steel syringe needles containing stylet wires were placed through the stoppers. Syringes were placed on the inserted needle to prevent any air water interaction.

2.5. Aromatic compound analysis by GC-MS

To quantify the volatile MAH compounds in the liquid phase tar, a GC-MS analysis method was developed. The following solvents were

tested for extraction: hexane, methanol, acetone, and dichloromethane (DCM). After preliminary testing, 100 mL of each solvent was added to 2 g of tar in 100 mL borosilicate bottles with butyl rubber/PTFE lined septa. Methanol and hexane did not dissolve all the tar, and soot or carbonaceous sorbents were still visible as black particles. DCM appeared to dissolve the butyl rubber septa and did not dissolve all the soot. In literature, often a mixture of hexane and acetone is mentioned as extraction solvent (Ghosh et al., 2000; Hodges and Richardson, 1996; Jonker and Koelmans, 2002; Jonker and Smedes, 2000; Lee, 2000). Testing a hexane-DCM mixture showed no proper mixing with the water phase and therefore was not suitable as solvent. However, the addition of hexane to the acetone did not show improved extraction over acetone alone. Based on the least solvent volume required to fully dissolve the tar including the soot, acetone was chosen as solvent.

The injected acetone extract volume was 10 μ L, with a split ratio of 1:6. The temperature program was set at: start 40 °C hold for 1 min, then to 200 °C at 10 °C/min and third step to 300 °C at 20 °C/min and hold 1 min. A Varian ms-column was employed, type VF-624, with dimensions 30 m \times 0.25 mm \times 1.40 μ m film thickness. Helium was used as carrier gas at 1 mL/min, and the injector temperature was set at 250 °C. For mass spectrometer, a Shimadzu QP-2010 plus was employed, mode EI/scan at a mass range of 29–300 amu. Deuterated compounds were used to show a high enough signal outside the natural mass distribution. Benzene- d_6 , toluene- d_8 , ethylbenzene- d_{10} , 1,3,5-trimethylbenzene- d_{12} and naphthalene- d_8 were purchased from Sigma-Aldrich and used as internal standards. External standards, containing all relatively highly water-soluble components from Pintsch gas tar were prepared as solutions in methanol as 2, 20, 50, 100 and 200 mg/L.

Gas chromatographic analyses of headspace analyses were performed on water samples from the tar to water partition tests on a Shimadzu GC-2010 and a PAL auto sampler. The same GC-MS method as for the acetone extracts was used. Injection volume was 250–500 μ L, with a split of 1:5–1:10. Samples were collected from monitoring wells A, B, C, D, E-1 and E-2 at the site (Fig. 1) and a GC-MS method was developed. The water samples were prepared and stored as described in Section 2.2.

2.6. Metabolite analysis using LC-qTOF MS

Analysis on metabolites was performed using liquid chromatography quadrupole time of flight mass spectrometry (LC-qTOF MS). To assess metabolites from mobile tar component degradation a target list

was compiled. Subsequently, a method was developed to qualify all targeted metabolites. For analysis, samples were sterilized with 25 mg/L of $HgCl_2$ to inhibit any biodegradation processes and were stored at -26 °C for a maximum of 180 days. Prior to analysis, 1.5 mL from the field sample was filtered and transferred into a 1.5 mL screw topped vial (VWR; cl. Gl. 32 \times 11.6 mm). Sample preparation is described in detail in the Supplementary information. For quantification, a standard mix was prepared containing 2-naphthoic acid, DL-benzylsuccinic acid, cinnamic acid, benzoic acid, 4-hydroxybenzoic acid and 1-H-indene-3-carboxylic acid. First, 1 g/L stock solutions were prepared by dissolving 10 mg of those compounds in 10 mL methanol. From the stock solutions, a mixture was prepared and used to make standard series containing; 1, 5, 10, 25, 50, 100, 200 and 500 μ g/L of each compound. A hybrid quadrupole orthogonal accelerated time-of-flight mass spectrometer, in combination with a Bruker maXis 4G mass spectrometer (MQ0201G053, Bruker Daltonics, Bremen, Germany), was used with a mounted HD collision cell for higher maximum resolution and sensibility on low molecular compounds (<200 m/z). The Shimadzu Nexera UHPLC system contained a SIL-30A autosampler, binary pumping system (LC-30AD) and a column oven (CTO-20AC) Shimadzu, Kyoto, Japan (Prantle 2014). An Acquity UHPLC CSHC18 column (150 mm \times 2.1 mm; 1.7 μ m; Waters) was used. Electrospray ionisation (ESI) was used to produce ions from macromolecules and compounds were measured in negative ionisation mode. A pre-run of 8 min was used for equilibration of the LC column, and sodium acetate (CH_3COONa), (0.25 mM in 1:1 isopropyl alcohol (IPA): H_2O) was used as calibration liquid. MS-MS fragments for the standards and suspects were searched by using Auto MS-MS data. Broadband CID (bbCID) was then used for suspect screening and quantification. A full description of the developed method is given in the Supplementary information.

For data acquisition and analysis, first the retention times of the standards were searched, based on their calculated exact masses, by using a software program DataAnalysis Compass IsotopePattern (omicX). Then, the recovery of the reference compounds was calculated from the spiked samples and MS-MS fragments were searched in DataAnalysis, using Mol-files from Chemspider. In the second phase, all data generated, including exact masses, retention times, and MS-MS fragments (and in some cases also ion ratios), were used to produce an automatic screening method in TASQ. Settings can be found in the description given in the Supplementary information.

All different topics for data acquisition and the method applied are included in Fig. 3.

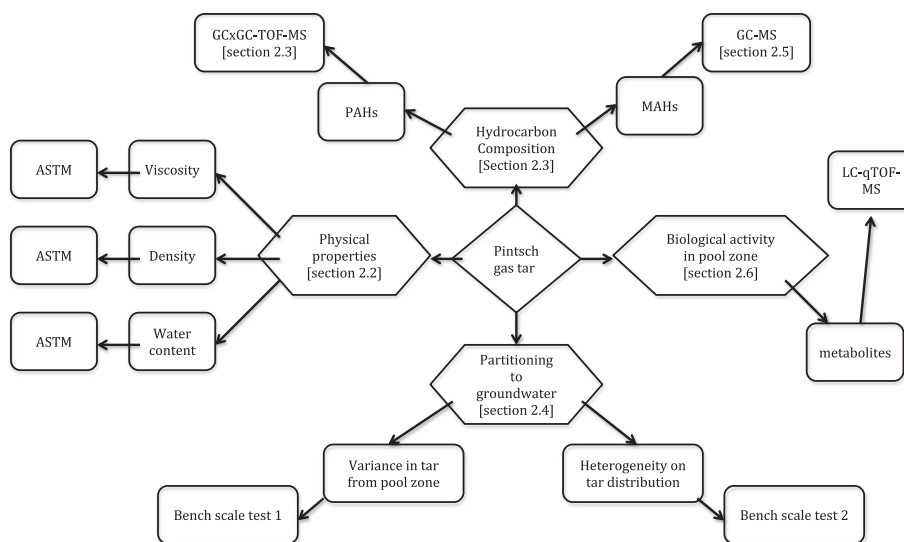


Fig. 3. Overview of used methods. In Section 2.2 methods are described for physical properties of tar samples. In Section 2.3 methods for determining tar composition are described. In Section 2.4 laboratory partitioning and dilution experiments are explained and in Section 2.6 methods leading to metabolite analyses are described.

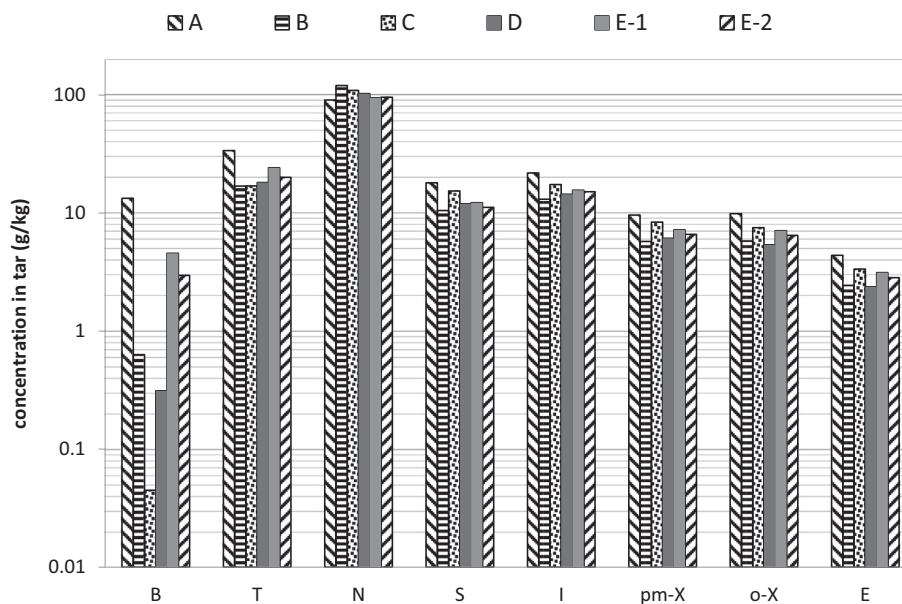


Fig. 4. Concentrations for water soluble compounds within Pintsch gas tar, using acetone extraction on pure phase tar and GC–MS analysis. Concentrations on y-axis represent concentrations in g/kg per compound in the liquid tar phase. X-axis represents measured compounds: B for benzene; T for toluene; N for naphthalene; S for styrene; I for indene; pm-X for para-/meta-xylenes; o-X for ortho-xylene; E for ethylbenzene; 2-MI for 2-methylindene; 1,2,4-TMB for 1,2,4-trimethylbenzene; 1-MN for 1-methylnaphthalene and 2-MN for 2-methylnaphthalene. No replicate measurements were conducted for this test.

3. Results

3.1. Pintsch gas tar composition

The Pintsch gas tar sample with the lowest viscosity and density was found closest to the former tar lagoon (Fig. 1, monitoring well A, Table 1). At this location, the tar pool height (6 m) is also the highest on-site, suggesting that the tar present here has the most original composition. In this tar sample, a total of 946 individual compounds were detected by GCxGC–TOFMS analysis. The large number of detected compounds illustrates the chemical complexity of the tar. This tar sample contained 57% polycyclic aromatic hydrocarbons (PAHs), 22% polycyclic aromatic sulphur heterocycles (PASH), 12% aliphatic compounds and alkylated PAHs, 6% polycyclic aromatic oxygen heterocycles (PAOH) and 2% polycyclic aromatic nitrogen heterocycles (PANH). A complete list of all detected components can be found in the Supplementary information. The primary compound was phenanthrene with 26.2 g/kg, followed by naphthalene, with 20.9 g/kg. A total of 57 alkylated benzenes ranging between C_9H_{12} and $C_{13}H_{20}$ were detected in the sample, with the largest number of isomers containing $C_{12}H_{18}$ with 17 isomers detected. The lowest molecular weight volatile aromatic compound

found was styrene. BTEX were not detected by the GCxGC–TOFMS, due to the analysis method. A relatively small number of 15 nitrogen-containing PAHs (PANHs) were detected, all in the form of 3- and 4-ring heterocycles. A total of 49 compounds representing oxygen-containing PAHs (PAOHs) were found, where the lowest molecular weight PAOH was three dibenzofuran isomers ($C_{18}H_{10}O_2$). A large number of sulphur-containing PAHs (PASHs) were detected with a total of 203 PASHs ranging from benzenethiol (C_6H_6S) to benzo[4,5]triphenylono [1,12-*bcd*]thiophene ($C_{20}H_{10}S$), phenanthro[3,4-*b*]thiophene, benzo [2,3]phenanthro[4,5-*bcd*]thiophene. No terpenes, such as known oil biomarkers hopanes or steranes, were detected in the Pintsch gas tar sample. Since BTEX components could not be detected by the GCxGC–TOFMS method used, additional analyses were performed by GC–MS.

The additional GC–MS analysis revealed light molecular hydrocarbon compounds from the Pintsch gas tar. Toluene (33.9 g/L) was the most abundant followed by xylenes (19.5 g/L), benzene (13.3 g/L), and ethylbenzene (4.4 g/L) (Fig. 4). Benzene, xylenes and toluene (BXT) content in our Pintsch gas tar sample was 6.7 wt%, which is relatively high compared 0.5 to 1 wt% for coal tars (Lee et al., 1992b; Peters and Luthy, 1993).

Table 2
calculated $\log K_{tw}$ and initial concentrations of water-soluble hydrocarbon compounds within the tar, using (Rixey et al.).

Compound	$\log K_{tw}$	$\log K_{ow}$	Solubility in water mg/L	A	B	C	D	E-1	E-2
	Calculated	Lit. ^a		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Benzene	2.3	2.1	1790	9649.2	409.9	61.9	436.4	4704.3	4348.8
Toluene	3.0	2.7	520	36,418.0	33,183.1	25,075.2	31,203.7	34,276.4	37,206.1
Ethylbenzene	3.5	3.2	150	6190.2	6660.5	7128.6	5730.7	6701.0	7556.9
m/p-Xylene	3.5	3.2	170	22,948.8	25,737.8	28,882.5	23,956.0	24,597.5	28,199.6
Styrene	3.0	3.1	300	10,436.9	12,316.2	13,544.9	11,250.4	11,235.3	12,750.3
o-Xylene	3.5	3.0	180	11,427.9	12,550.8	12,891.1	9980.0	12,084.0	13,717.9
3-Ethyltoluene	4.1	3.6	40	4567.1	4988.9	4815.9	4133.3	5261.1	6143.5
1,2,4-Trimethylbenzene	4.2	3.8	60	16,681.3	18,636.9	17,572.2	15,035.4	19,188.3	22,592.8
1,2,3-Trimethylbenzene	4.2	3.7	60	6641.2	6917.5	6318.1	5381.4	7188.0	8731.9
Indene	3.5	2.9	20	21,170.5	25,968.8	25,464.1	21,255.6	24,675.1	32,692.6
2-Methylindene	4.0	2.6	20	9064.2	9364.8	8255.3	7646.7	9233.5	10,534.2
Naphthalene	4.1	3.3	30	221,150.2	243,693.4	289,798.6	274,428.5	236,276.9	244,929.1
Total				376,345.3	400,428.7	439,808.4	410,437.9	395,421.5	429,403.7

^a $\log K_{ow}$ values from national centre of biotechnology.

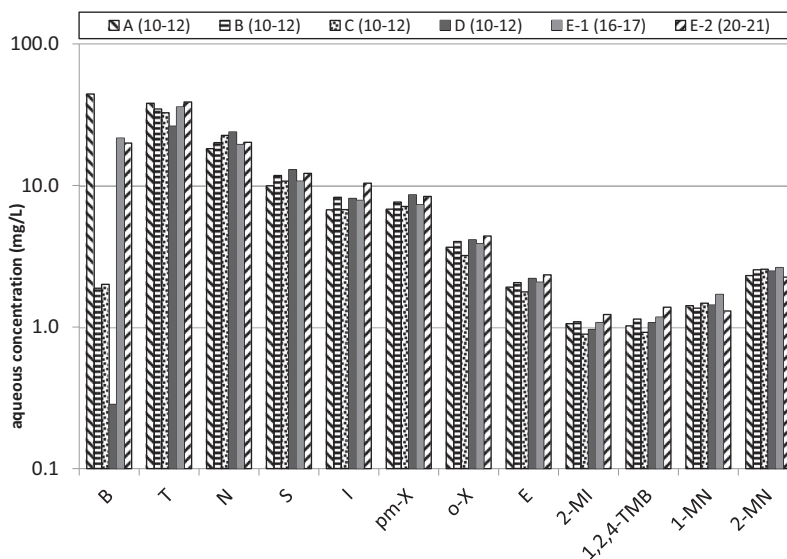


Fig. 5. Concentration of water soluble hydrocarbon compounds partitioning from tar to the water phase at a ratio of 1 volume of tar to 4 volumes of water (tar:water = 1:4). Concentrations are given in mg/L per compound in the aqueous phase. Tar samples were collected from 6 monitoring wells (A, B, C, D, E-1, E-2) at varying location from the pool zone. X-axis represents measured compounds: B for benzene; T for toluene; N for naphthalene; S for styrene; I for indene; pm-X for para-/meta-xylenes; o-X for ortho-xylene; E for ethylbenzene; 2-MI for 2-methylindene; 1,2,4-TMB for 1,2,4-trimethylbenzene; 1-MN for 1-methylnaphthalene and 2-MN for 2-methylnaphthalene. No replicate measurements were conducted in this test.

3.2. Partitioning of tar to water from several samples across the pool zone

Four liquid-phase tar samples from different locations in the source zone (A, B, C and D, Figs. 1 and 2) were analysed to compare the tar composition and partitioning behaviour after decades in the aquifer.

Twelve MAHs components were measurable in the water phase (Table 2), of which the concentrations of benzene, toluene and naphthalene were highest. Additionally, in the water extracts from two samples from the deep aquifer (E-1 and E-2), iso-propylbenzene, also known as cumene, and 1,2,4,5-tetramethylbenzene were identified. Water extracts from the four tar samples from the pool zone (A, B, C, D) show a decrease of trend of decreasing concentration of benzene and toluene

concentrations. The decrease in benzene corresponds to an increasing distance from the former tar lagoon (Fig. 5). The benzene concentration in the water extracts decreased from 44.3 mg/L in well A to 0.3 mg/L in well D, which is located 70 m from the entry point of the DNAPL source. In contrast, concentrations of other relatively water-soluble components, such as styrene, indene, xylenes and ethylbenzene, did not decrease in the extracts from downstream samples (Table 2 and Fig. 5). Naphthalene concentrations showed an inverse relationship to benzene, with increasing concentrations with distance from the source entry point; the naphthalene concentration in well A was 18.2 mg/L, while 70 m towards the pool fringe in well D the concentration was 23.9 mg/L (Fig. 5).

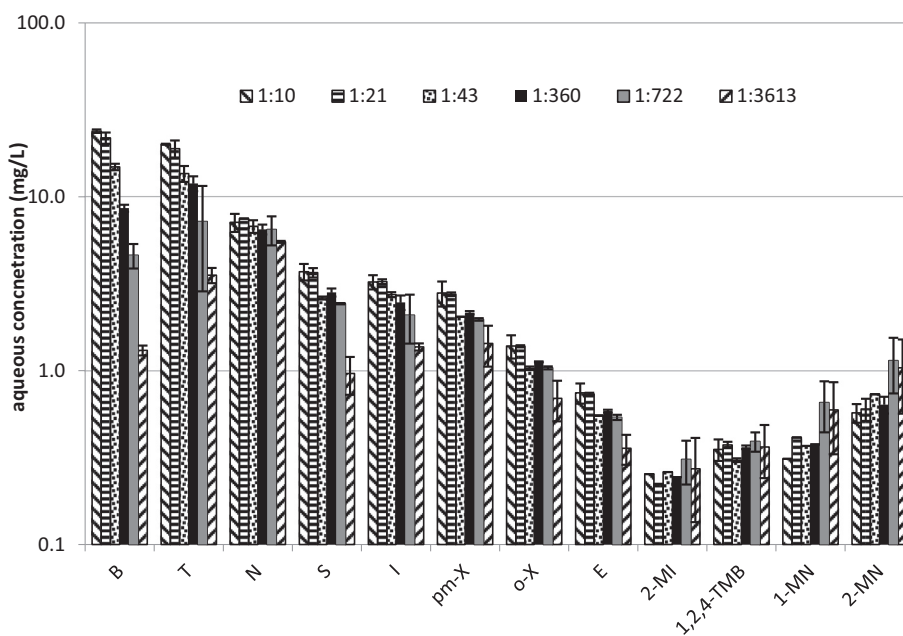


Fig. 6. Concentration of water-soluble hydrocarbon compounds partitioning from tar to the water phase at increasing water volumes. Concentrations are given in mg/L per compound in the aqueous phase. The most original tar from site was used in this test, collected from well A at presumed historical entry point. X-axis represent measured compounds: B for benzene; T for toluene; N for naphthalene; S for styrene; I for indene; pm-X for para-/meta-xylenes; o-X for ortho-xylene; E for ethylbenzene; 2-MI for 2-methylindene; 1,2,4-TMB for 1,2,4-trimethylbenzene; 1-MN for 1-methylnaphthalene and 2-MN for 2-methylnaphthalene. Error bars represent standard deviations based on analysis from duplicate test samples.

3.3. Dilution effects of aromatic hydrocarbons partitioning

The effects of increasing dilution on the partitioning behaviour of tar components were assessed in a series of tar-water dilution batch experiments (Fig. 6). Tar from well A (Fig. 1) was exposed to increasing water volumes (tar:water ratios, v:v). In the tar water extracts, 18 lower molecular weight aromatic hydrocarbon components were detected by GC-MS analysis. Benzene was the lowest detected molecular weight aromatic hydrocarbon compound and 1- and 2-methylnaphthalene were the heaviest detected aromatic components. In addition, compounds not commonly associated with tar contaminated sites were detected, such as styrene, ethyltoluenes, diethylbenzene, 1,2,4,5-tetramethylbenzene and methylindenes.

Changes in aromatic hydrocarbons leaching from the tar into the increasing groundwater volumes were observed (Fig. 6). In samples with water to tar ratio of 10:1 and 21:1, approximately the same total concentrations of dissolved aromatic hydrocarbons were found, 63.4 mg/L and 64.9 mg/L, respectively. This similarity indicates that the maximum solubility of dissolved hydrocarbons was reached in the water phase. The concentrations and composition of the water-soluble hydrocarbons changed at more diluted water to tar from >43:1 up to 3613:1 (Fig. 6). The total concentration of detected hydrocarbons dropped from

64.9 mg/L to 23.3 mg/L for diluted water to tar from 21:1 to 3613:1. Benzene showed the largest aqueous concentration decrease when diluted at higher dilutions. This can be explained by possible depletion of benzene in the tar. Aqueous concentration decrease was not in the order of high to low water solubility but varied due to initial concentrations in the tar. Benzene was followed by toluene, styrene, indene, naphthalene, xylenes and ethylbenzene. For methylindene and 1,2,4-trimethylbenzene, no concentration changes were detected. Dissolved 1- and 2-methylnaphthalene concentrations increased when more groundwater was present, from 0.3 mg/L and 0.6 mg/L to 0.6 mg/L and 1.0 mg/L respectively, (Fig. 6).

3.4. Metabolites detected from Pintsch gas tar

To assess the occurrence of biodegradation, the aqueous phase from the tar-water partition vials was screened for metabolites. In total, 46 specific metabolites were detected (Table 3). The identified metabolites were divided into signature-, suspect- and proposed metabolites. Signature metabolites originate from one parent compound, while a suspect metabolite could be originating from several parent compounds and a proposed metabolite is a potential metabolite that has not been described as one so far to our knowledge. Parent compounds relating to

Table 3
Overview of detected metabolites from Pintschs gas tar. Pintsch gas tar samples obtained from a liquid DNAPL pool zone in a shallow aquifer. Metabolites are categorized in signature-, suspect-, and proposed metabolites. Parent compound(s) are given for each metabolite, including references and specific remarks.

Category	Metabolite	Parent compound	Reference/remark
Suspect	Benzoic acid	Benzene, toluene	(Callaghan, 2013)
Suspect	Cinnamic acid	Toluene	(Chee-Sanford et al., 1996)
Suspect	Phenylsuccinic acid	Toluene	(Leutwein and Heider, 2001)
Signature	Benzylsuccinic acid	Toluene	(Callaghan, 2013)
Signature	Benzoylactic acid	Ethylbenzene	(Callaghan, 2013)
Signature	3-o-Toluoyl propionic acid	Xylenes	(Morasch et al., 2004)
Signature	Methylbenzylsuccinic acid	Xylenes	(Beller et al., 1996; Young and Phelps, 2005)
Suspect	Acetylphenol	m-Xylene, styrene	(Tischler, 2015)
Suspect	Hydroxyphenylacetic acid	Styrene	(Tischler, 2015)
Signature	Dimethyl-benzoic acid	Trimethylbenzene	(Cozzarelli et al., 1990)
Proposed	Indyl methyl succinic acid	Indene	-
Signature	Indenediol	Indene	(Kim et al., 2011)
Signature	1H-indene-2-carboxylic acid	Indene	(Callaghan, 2013)
Signature	Dihydro-2-indenoic acid	Indene	(Callaghan, 2013)
Proposed	Tetrahydro-2-indenoic acid	Indene	-
Proposed	Dihydromethyl indenoic acid	Indene, naphthalene	-
Suspect	Octahydro-2-naphthoic acid	2-Naphthoic acid	(Ohlenbusch et al., 2002; Zhang et al., 2000)
Suspect	2-Carboxycyclohexylacetic acid	Oxo-decahydro-2-naphthoic acid	(Safinowski, 2005; Zhang et al., 2000)
Suspect	Methyl benzoic acid	Naphthalene	(Safinowski and Meckenstock, 2006)
Signature	1-Naphthoic acid	Naphthalene	(Zhang et al., 2000)
Signature	2-Naphthoic acid	Naphthalene	(Zhang et al., 2000)
Suspect	Naphthaleneacetic acid	Naphthalene	(Safinowski and Meckenstock, 2006)
Suspect	2,3-Naphthalenedicarboxylic acid	Anthracene	(Richnow et al., 1999)
Suspect	Naphthyl-2-methyl-succinate	Naphthalene	(Annweiler et al., 2000)
Suspect	P-cresol	Phenanthrene	(Tsai et al., 2009)
Suspect	4-Hydroxy benzoic acid	Phenanthrene, benzene	(Foght, 2008; Tsai et al., 2009)
Suspect	Octanoate	Octanoate	(Bian et al., 2015)
Suspect	Adipic acid	Cyclohexane	(Verschueren, 2001)
Suspect	4-Hydroxyphenylpyruvate	Phenylacetate	(Mohamed et al., 1993)
Suspect	Phenylglyoxylic acid	Phenylacetate, phenol, benzene	(Leibman, 1975; Mohamed et al., 1993; Tischler, 2015; Tischler and Kaschabek, 2012)
Suspect	Butyrate	Alkanes	(Bian et al., 2015)
Suspect	Nonanoate	Alkanes	(Bian et al., 2015)
Suspect	2-(1-Methyldodecyl)succinate	Alkanes	(Bian et al., 2015)
Suspect	2-(1-Methyltetradecyl)succinate	Alkanes	(Bian et al., 2015)
Suspect	Methylmalonate semialdehyde	Hydrocarbon derivative	The Metabolomics Innovation Centre
Suspect	Methylglyoxal	Acetone	(Bellier et al., 2019)
Suspect	2-Methyl 2-hydroxy-propanal	Acetone	(Frey et al., 2016)
Suspect	Valeric acid or pentanoic acid	-	Saturated fatty acid
Suspect	Fumaric acid	Maleic acid, succinate	Dicarboxylic acid, (Callaghan et al., 2012)
Suspect	Succinic acid	Propionate	Dicarboxylic acid involved in succinilation of aromatic compounds (Callaghan et al., 2012)
Suspect	3-OH-isovaleric acid	Butyrate, isovaleric acid	PubChem
Suspect	Quinolinic acid	-	Production of nicotinamide adenine dinucleotide (NAD+)
Suspect	Hippurate	Toluene, benzene, benzoic acid	Reported in urine of workers exposed to toluene (Hasegawa et al., 1983)
Suspect	Ethylmalonate	Malonic acid	Dicarboxylic acid, and a product of the skeleton rearrangement (Bian et al., 2015)
Suspect	Butylmalonate	Malonic acid	Dicarboxylic acid and a product of the skeleton rearrangement (Bian et al., 2015)
Suspect	2-Isopropylmalic acid	a-Ketoisovaleric acid	An intermediate in the Leucine biosynthesis of proteins (Strassman and Ceci, 1963)

these signature metabolites were toluene, ethylbenzene, xylenes, trimethylbenzene, indene and naphthalene (Table 3). Furthermore, 34 suspect metabolites were detected. These suspect metabolites related to biodegradation of BTEX, styrene, indene, naphthalene, phenanthrene and anthracene (Table 3). Moreover, metabolites were detected relating to biodegradation of cyclohexane, alkanes and acetone. Furthermore, several dicarboxylic acids were detected and metabolites that relate to carbon skeleton rearrangement of hydrocarbon compounds.

In the core of the tar pool zone (well A), the highest number of metabolites were present (42 compounds). In samples near the fringe of the pool zone less metabolites were found to be present, varying from 40 metabolites in well B, 41 in well C and 37 in well D. In samples from the deep aquifer 38 and 37 metabolites, were detected from well E-1 and well E-2 respectively. In the groundwater reference sample, which is located upgradient, 16 metabolites were detected.

Out of all targeted metabolites, five were quantified using standards: butyric, cinnamic, benzoic, 1H-indene-2-carboxylic- and 2-naphthoic acid. The compound confidence scores and concentrations of the detected metabolites are given in the Supplementary information. One of the metabolites detected upgradient in the reference sample was benzoic acid. Benzoic acid is a suspect metabolite for various mono aromatic hydrocarbons. However, the detected concentration upgradient was relatively low 14.6 µg/L, compared to 231.7–309.5 µg/L detected in the tar samples. Making the upgradient concentration 15 times lower than the lowest concentration of the six tar samples (231.7 µg/L).

Signature metabolite for anaerobic indene degradation, 1-H-indenecarboxylic acid was detected in all tar samples. Concentrations for 1-H-indenecarboxylic acid ranged from 0.8 to 1.2 µg/L. Cinnamic acid a suspect metabolite for toluene was found at concentrations as high as 3.0 µg/L and 2-naphthoic acid a metabolite for naphthalene up to 6.6 µg/L. The upgradient reference sample revealed no 1-H-indenecarboxylic acid, cinnamic acid, nor naphthoic acid.

Additionally, several suspect metabolites were detected, such as phenyl-, benzyl- and methylbenzyl-succinic acid, ethylmalonate, acetylphenol, butylmalonate and isopropylmalonate (Table 3).

Compounds, indyl methyl succinic acid and tetrahydro-2-indenoic acid, were recognized as potential metabolites for indene. These metabolites were nominated as proposed metabolites. The detected signature, suspect and (new) proposed metabolites for indene were combined, and introduced as an anaerobic biodegradation pathway for indene, following the carboxylation process (Fig. 8).

Amongst the detected components also fatty acids and dicarboxylic acids were found to be present, such as: butyric-, valeric-, fumaric-, succinic-, 3-OH-isovaleric-, octanoic-, adipic-, cinnamic-, nonanoic-, quinolinic- and hippuric acid. These compounds were not detected in the reference sample; however, they are known products of hydrocarbon fermentation (Table 3).

4. Discussion

4.1. Composition of Pintsch gas tar

In the analysed Pintsch gas tar, phenanthrene was found to be the most dominant compound, while in coal tar this is commonly naphthalene (Brown et al., 2006; Electric Power Research Institute, 1993). The detected n-alkane distribution is most similar to diesel as can be seen from Fig. 7, (Coulon et al., 2004; Kaplan et al., 1997), suggesting that the original feedstock was similar to diesel oil. The absence of hopanes and steranes in the Pintsch gas tar suggests that the feedstock was a refined product and supports that diesel-like oil has been used. An alternative possibility is that the Pintsch gas production process itself could be responsible for loss of hopanes and steranes, due to the high temperatures involved. Although few alkylated benzenes were expected to be present within Pintsch gas tar in relation to the production process (Lunge, 1909), we found that alkylated benzenes make up a notable group of compounds. In addition, 15 PANHs compounds were detected

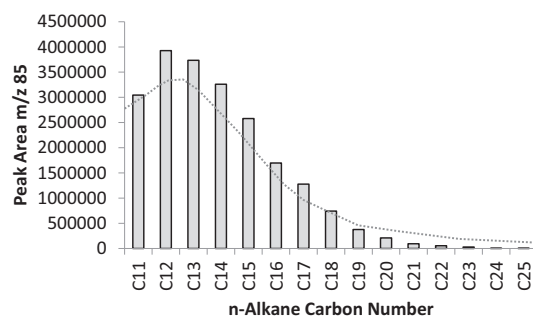


Fig. 7. Distribution pattern of n-alkanes for Pintsch gas tar (sample well A) and reference distribution pattern diesel represented as a dotted line from (Coulon et al., 2004). No replicate measurements were conducted for this test.

in the form of heterocycles; this is a relatively small number and likely due to a low nitrogen content in the feedstock oil (<0.5%) compared to coal tar (>0.5–2%) (Burchill et al., 1983). Our findings indicate that Pintsch gas tar contains a relatively high content of BTX and other MAH compared to known coal tars (Brown et al., 2006; Electric Power Research Institute, 1993). BTEX in tar is usually close to 0.5 wt% (Peters and Luthy, 1993). Due to relatively high concentrations partitioning into the water phase, it was calculated that BTEX and naphthalene make up 36.5 wt% of the most original tar found on the site. The naphthalene content in Pintsch gas tar ($\pm 21\%$) is relatively high compared to coal tar (10.5%) (D’Affonseca et al., 2011). High BTEX concentrations can also be substantiated as benzene and toluene were the main components of the lighting gas produced by the Pintsch gas process (Butterfield, 1904). The weight percentage of MAH compounds in tar is important because they affect the viscosity of the tar (Electric Power Research Institute, 1993). The tar samples obtained in this study north of the former tar lagoons show higher viscosity than found at the bulk of the pool situated directly beneath the former tar lagoon. This suggests substantial depletion of BTEX from the migrated tars in this study (Table 1).

4.2. Physical properties and partitioning behaviour

The tar-water extraction experiment shows the highest concentrations of benzene and toluene in the water phase (Fig. 6). Benzene and toluene concentrations decreased at increasing water volumes. The tars sampled from different locations in the pool zone, show similar partitioning behaviour after extraction with water. Samples from the shallow aquifer show that benzene and toluene concentrations decrease along the path of migration over the dipping aquitard, as seen in the dilution experiment. The most original tar shows the highest partitioned benzene concentration and least dilution. The tar that travelled the furthest and was sampled near the tar pool fringe, showed the lowest concentration of benzene and the most dilution. Since groundwater flow is opposite to the dipping direction of the aquitard, this indicates that the tar is more depleted due to dissolution along the tar migration path; the dilution experiment simulates the field situation well. While the concentrations decreased for benzene and toluene, it is not so for other constituents such as styrene, indene, xylenes and ethylbenzene. Concentrations of these constituents in water decreased with increasing water volumes in the experiment (Fig. 6) as expected from partitioning calculations, but this was not observed in the samples from the pool zone (Fig. 5), with distance from the tar lagoon. An explanation could be that due to biodegradation of benzene and toluene in the water phase, their dissolution is enhanced, and they become depleted in the tar whereas this is not the case for the other components. The viscosity of the tar samples ranged from 9.72 to 18.25 cP at 25 °C, which is relatively low compared to values given in the literature for coal tar samples, ranging from 34 to 6000 cP at 40 °C (Lee et al., 1992a; Peters and Luthy, 1993). This points to the enhanced dissolution

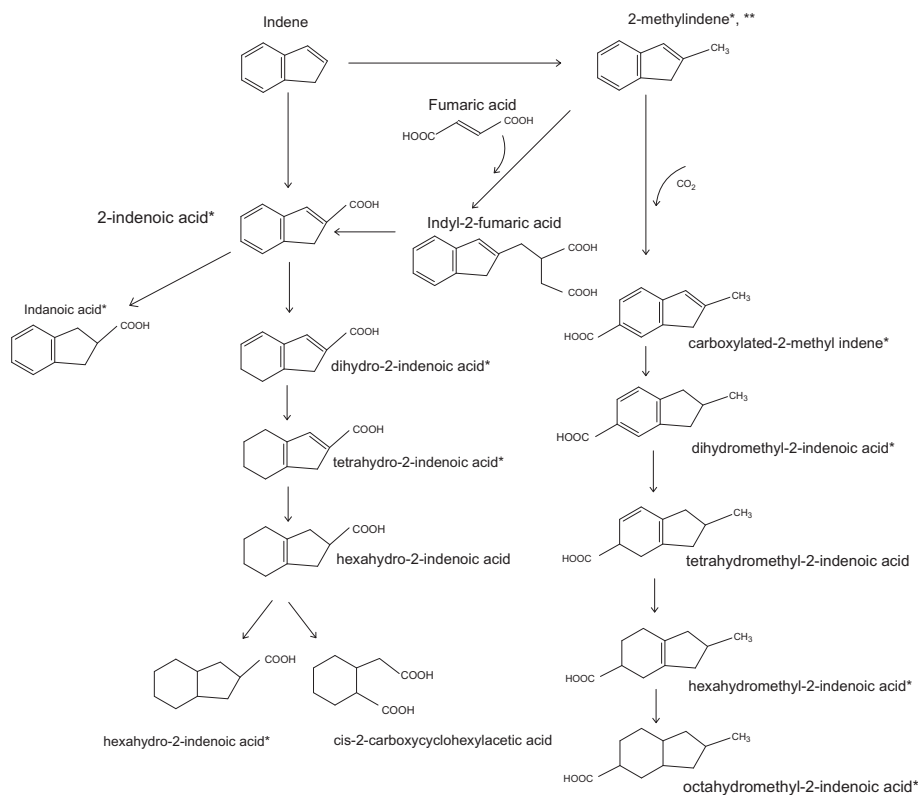


Fig. 8. Introduced anaerobic indene biodegradation pathway; * = detected compound, ** = possible metabolite, but can also be originating from Pintsch gas tar.

and depletion of the MAH compounds along the migration path of tar. Thus, the most soluble and degradable aromatics are more rapidly depleted.

The determination of tar composition using acetone extraction, showed higher concentrations for all water-soluble components in the tar compared to calculated weight percentages from aqueous concentrations using Raoult's law. This indicates partition behaviour differences for acetone and water. The mass fractions in the tar were higher for the lightest molecular weight compound, benzene, up to four times higher than that of methylindene. A similar effect has been described by Peters and Luthy (1993), where naphthalene concentrations in water, were in line with Raoult's law predictions, but higher

molecular compounds, such as phenanthrene and pyrene, showed higher than predicted concentrations. Ideal behaviour is not necessarily expected for tar water partitioning and a factor of two is accepted for field-scale approximation of partitioned concentrations due to significant errors (Lee et al., 1992b). Due to the high content of MAH in Pintsch gas tar, the non-ideal behaviour could be greater for higher molecular weight components. Characterization of tars is known to be difficult due to the large number of constituents. Different extracts from tar generally have the same functional groups but in different concentrations depending on the solvent used. This is due to the variance in the ability of solvents to extract components with certain functional groups (Guillén et al., 1991). Solvents with a high density and low viscosity

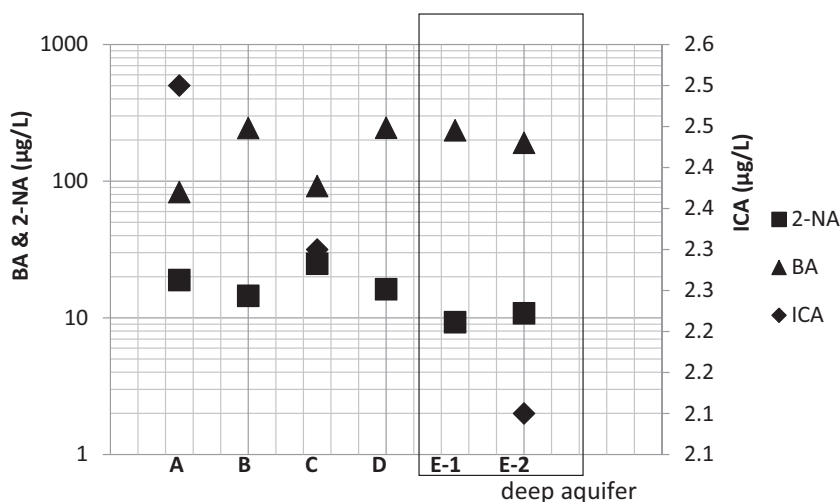


Fig. 9. Aqueous concentration of metabolite concentration for BA (benzoic acid), ICA (indene carboxylic acid), 2-NA (2-naphthoic acid) from Pintsch gas tar samples. No replicate measurements were conducted in this test.

tend to produce more reproducible data. However, there is no simple relationship between extraction yield and the ability of a solvent to interact with a tar. Extraction yields for BTEX and naphthalene from coal tar using varying solvents may show a difference of up to 14% for hexane, 20% for ethanol, 49% for acetone and 64% for DCM (Guillén et al., 1991). For Pintsch gas tar, acetone was chosen as the solvent due to its ability to dissolve the total tar mass, without leaving soot, which did occur with hexane, ethanol, and DCM.

4.3. Biodegradation indicators within the source zone

It is known that anaerobic hydrocarbon degradation of crude oil is a common process in relatively shallow, but still in the deep subsurface, oil reservoirs (Aitken et al., 2004). The presence of biomarkers and compound-specific signature metabolites extracted from crude oil is one line of evidence to show the occurrence of anaerobic biodegradation (Bian et al., 2015). Metabolite profiling in a tar contaminated shallow aquifer, and relatively shallow subsurface, could also be used as a line of evidence of anaerobic biodegradation. The presence of 46 putative metabolites detected in the Pintsch gas tar samples suggests active biodegradation in the liquid DNAPL containing source zone. The results show that liquid phase tar samples collected from both the shallow and the deep aquifer hold a variety of metabolites. Where the most metabolites are present in the core of the tar pool and less metabolites are present in the proximity of nitrate-reducing groundwater conditions, at the fringe of the tar pool. Although most metabolites had a high identification confidence score (Schymanski et al., 2014), and appeared at similar retention times in different samples, the identification of metabolites is not definite. The interpretation is mostly based on MS/MS fragmentation, rather than on comparison with reference standards (Krauss et al., 2010). Reference standards are unfortunately not available for most detected metabolites.

Indene carboxylic acid, a signature metabolite for carboxylation of indene (Callaghan, 2013), and indanoic acid a suspect metabolite for indane or indene (Callaghan, 2013), were detected in several tar samples. Other compounds were recognized as metabolites from indene, due to their indene associated molecular structure in combination with a carboxylate. Indene carboxylic acid is described as a possible first intermediate in the degradation of indene or 2-methylindene (Callaghan, 2013; Safinowski, 2005). Followed by dihydro-2-indenoic acid down to hexahydro-2-indenoic acid or cis-2-carboxycyclohexylacetic acid (Fig. 8). The new introduced metabolic pathway, suggests, indene is reduced stepwise before ring cleavage occurs. This stepwise reduction is similar to that of anaerobic naphthalene biodegradation following the carboxylation pathway (Meckenstock et al., 2000).

2-Naphthoic acid (2-NA) and benzoic acid (BA) concentrations (Fig. 9) are relatively high compared to previously found in contaminated groundwater plumes (Jobelius et al., 2011; Ohlenbusch et al., 2002). This suggests that these metabolites respectively from naphthalene or benzene, toluene, ethylbenzene, xylenes, although formed in the aqueous phase accumulated in the tar at this site. The highest concentrations of BA were found in tar extracts from wells B and D. These wells are situated to the north of the former tar lagoon, where groundwater from upstream, containing nitrate and sulphate, meets the fringe of the DNAPL. The highest 2-NA and indene carboxylic acid (ICA) concentrations were detected in wells A and C, situated towards the middle of the pool zone, where less or no nitrate and sulphate are present in the surrounding groundwater. This suggests that at the upgradient side of the pool zone, relatively more BTEX is biodegraded and further downgradient and deeper in the source zone, relatively more naphthalene and indene were biodegraded.

All detected metabolites were extracted from tar that had been in the subsurface for approximately 60 to 100 years. Presence of metabolites from anaerobic biodegradation could be explained by:

1. Formation during the invasion of tar in the subsurface and remained within the tar for decades.
2. Biodegradation in the source zone is active and metabolites are formed in the vicinity of the tar and once formed in the groundwater these metabolites (re-)dissolve into the tar.
3. Metabolites originate from the original distilled feedstock prior to the Pintsch gas process.

All detected metabolites could potentially originate from crude oil, distilled feedstock, or the Pintsch gas tar itself. However, it is unlikely that carboxylated hydrocarbon compounds survived the distillation process from crude oil to feedstock (diesel/gasoil), as temperatures during production would have been 700–1000 °C (Butterfield, 1904). Benzoic acid and naphthoic acid are known to be thermally destroyed at 530–762 °C and 305–410 °C, respectively (Brzyska and Kula, 1995; Winter et al., 1969). Therefore, it is likely that benzoic acid, naphthoic acid and possibly all other found metabolites are formed in the aquifer and indicate biodegradation of hydrocarbons in the source zone. When metabolites are formed at the tar-water interface and/or in groundwater adjacent to pure phase tar, the metabolites can either be transported downstream by groundwater advection, or the metabolites remain in the source zone, because they (re-)dissolve back from the aqueous phase into the tar as they are still relatively hydrophobic. When the sum of all detected metabolites is compared to the sum of the water-soluble monocyclic and polycyclic aromatic hydrocarbon concentrations, we find that the least metabolites are present where the water-soluble hydrocarbons have the highest concentration (Fig. 10).

5. Conclusions

Although Pintsch gas tar produced from oil can be mistaken for coal tar, we found that phenanthrene is the most abundant constituent rather than naphthalene and holds a high MAH content. The n-alkane distribution also shows high similarity to that of diesel. The low viscosity and low density of Pintsch gas tar originates from a relatively high content of MAH compounds. Therefore, tar produced by the Pintsch gas process potentially has relatively high invasive properties in the subsurface and partitioning characteristics to the groundwater. MAH compounds, such as styrene, ethyltoluenes, diethylbenzene, trimethylbenzenes and 1,2,4,5-tetramethylbenzene, are not commonly reported at tar contaminated sites, however, these compounds are present within Pintsch gas tar. These MAHs are soluble in groundwater, partition from the tar into groundwater and potentially are present in contaminant plumes.

The abundance of biomarkers found within Pintsch gas tar samples from the study site suggests active anaerobic biodegradation under the pool zone conditions. A considerable amount of indene related metabolites was detected that can be attributed to anaerobic biodegradation by the carboxylation pathway. From these ten indene related metabolites, an anaerobic carboxylation pathway is introduced.

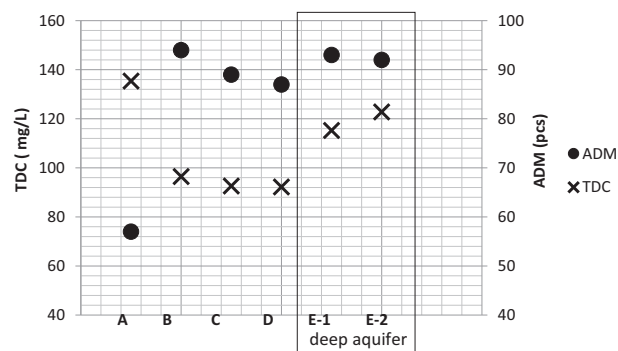


Fig. 10. Total of all detected metabolites and their isomers (ADM) and total aqueous concentration of the detected water-soluble hydrocarbon compounds (TDC). No replicate measurements were conducted in this test.

The metabolites present in the tar can be used as biomarkers for active biodegradation under pool zone conditions. The geochemical and biological conditions at the investigated former Pintsch gas plant are representative for other contaminated sites. We therefore suggest that the detected metabolites from biodegradation under source zone conditions also occur at other hydrocarbon contaminated sites. The partitioning of contaminants from multi-component DNAPL into the aqueous phase and re-dissolution of their slightly less hydrophobic metabolites back from the aqueous phase into the DNAPL is feasible and demonstrates the complexity of assessing degradation processes within a source zone.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

We thank the Stichting Bodemsanering NS (SBNS), for funding the research on partitioning experiments, and metabolite screening on LC-qTOFMS. We also, we like to thank the Scottish Funding Council (SFC) Glasgow Research Partnership in engineering, the University of Strathclyde, WSP and National Grid Property for funding support for the GCxGC MSTOF analysis of the Pintsch gas sample. We thank Andre Cinjee (Deltares) and Patrick Broekhuizen (Aveco de Bondt) for their assistance in the field work and Frederic (Deltares) for her assistance in the laboratory. We also thank Alexandra Hockin (KWR) for her experimental and English grammar contributions during her internship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.137797>.

References

- Aitken, C.M., Jones, D.M., Larter, S.R., 2004. Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs. *Nature* 431, 291–294. <https://doi.org/10.1038/nature02922>.
- American, S., 1898. The manufacture of Pintsch gas. *Sci. Am.* LXXIX, 27.
- Annweiler, E., Richnow, H.H., Antranikian, G., Hebenbrock, S., Garms, C., Franke, S., Francke, W., Michaelis, W., 2000. Naphthalene degradation and incorporation of naphthalene-derived carbon into biomass by the thermophile *Bacillus thermoleovorans*. *Appl. Environ. Microbiol.* 66, 518–523. <https://doi.org/10.1128/AEM.66.2.518-523.2000>.
- Beller, H.R., Spormann, A.M., Sharma, P.K., Cole, J.R., Reinhard, M., 1996. Isolation and characterization of a novel toluene-degrading, sulfate-reducing bacterium. *Appl. Environ. Microbiol.* 62, 1188–1196.
- Bellier, J., Nokin, M.J., Lardé, E., Karoyan, P., Peulen, O., Castronovo, V., Bellahcène, A., 2019. Methylglyoxal, a potent inducer of AGEs, connects between diabetes and cancer. *Diabetes Res. Clin. Pract.* 148, 200–211. <https://doi.org/10.1016/j.diabres.2019.01.002>.
- Bian, X.-Y., Mbadinga, S.M., Liu, Y.-F., Yang, S.-Z., Liu, J.-F., Ye, R.-Q., Gu, J.-D., Mu, B.-Z., 2015. Insights into the anaerobic biodegradation pathway of n-alkanes in oil reservoirs by detection of signature metabolites. *Sci. Rep.* 5, 9801. <https://doi.org/10.1038/srep09801>.
- Birak, P.S., Miller, C.T., 2009. Dense non-aqueous phase liquids at former manufactured gas plants: challenges to modeling and remediation. *J. Contam. Hydrol.* 105, 81–98. <https://doi.org/10.1016/j.jconhyd.2008.12.001>.
- Brown, D.G., Gupta, L., Kim, T.H., Keith Moo-Young, H., Coleman, A.J., 2006. Comparative assessment of coal tars obtained from 10 former manufactured gas plant sites in the eastern United States. *Chemosphere* 65, 1562–1569. <https://doi.org/10.1016/j.chemosphere.2006.03.068>.
- Bruce, L., Miller, T., Hockman, B., 1991. Solubility versus equilibrium saturation of gasoline compounds: a method to estimate fuel/water partition coefficients using solubility and Koc. *NWWA/API Conf. Pet. Hydrocarb. Gr. Water.* 843 (13), 369–411. <https://doi.org/10.1201/es60085a010>.
- Brzyska, W., Kula, A., 1995. Studies on the thermal decomposition of rare earth element complexes with 2-naphthoic acid. *J. Therm. Anal. Calorim.* 44, 1159–1169. <https://doi.org/10.1007/BF02547546>.
- Burchill, P., Herod, A.A., Pritchard, E., 1983. Investigation of nitrogen compounds in coal tar products. 1. Unfractionated materials. *Fuel* 62, 11–19. [https://doi.org/10.1016/0016-2361\(83\)90245-4](https://doi.org/10.1016/0016-2361(83)90245-4).
- Butterfield, W.J., 1904. *The Chemistry of Gas Manufacture*. Edition 3. Charles Griffin and Company, Exeter.
- Callaghan, A.V., 2013. Metabolomic investigations of anaerobic hydrocarbon-impacted environments. *Curr. Opin. Biotechnol.* 24, 506–515. <https://doi.org/10.1016/j.copbio.2012.08.012>.
- Callaghan, A.V., Morris, B.E.L., Pereira, I.A.C., McInerney, M.J., Austin, R.N., Groves, J.T., Kukor, J.J., Sufliata, J.M., Young, L.Y., Zylstra, G.J., Wawrik, B., 2012. The genome sequence of *Desulfatibacillum alkenivorans* AK-01: a blueprint for anaerobic alkane oxidation. *Environ. Microbiol.* 14, 101–113. <https://doi.org/10.1111/j.1462-2920.2011.02516.x>.
- Chee-Sanford, J.C., Frost, J.W., Fries, M.R., Zhou, J., Tiedje, J.M., 1996. Evidence for acetyl coenzyme A and cinnamoyl coenzyme A in the anaerobic toluene mineralization pathway in *Azoarcus toluolyticus* Tol-4. *Appl. Environ. Microbiol.* 62, 964–973.
- Coulon, F., Pelletier, E., St. Louis, R., Gourhant, L., Delille, D., 2004. Degradation of petroleum hydrocarbons in two sub-Antarctic soils: influence of an oleophilic fertilizer. *Environ. Toxicol. Chem.* 23, 1893–1901. <https://doi.org/10.1897/03-484>.
- Cozzarelli, I.M., Eganhouse, R.P., Baedecker, M.J., 1990. Transformation of Monoaromatic hydrocarbons to organic acids in anoxic groundwater environment. *Environ. Geol. Water Sci.* 16, 135–141. <https://doi.org/10.1007/BF01890379>.
- D'Affonseca, F.M., Prommer, H., Finkel, M., Blum, P., Grathwohl, P., 2011. Modeling the long-term and transient evolution of biogeochemical and isotopic signatures in coal tar-contaminated aquifers. *Water Resour. Res.* 47, 1–22. <https://doi.org/10.1029/2010WR009108>.
- Egloff, G., Twomey, T.J., 1916. The effect of temperature on the formation of benzene, toluene, xylene, naphthalene and anthracene from petroleum at atmospheric pressure. *J. Phys. Chem.* 20, 121–150.
- Electric Power Research Institute, 1993. *Chemical and Physical Characteristics of Tar Samples From Selected Manufactured Gas Plant (MGP) Sites; Final Report for Research Project 2879-12; EPRI TR-102184*.
- Foght, J., 2008. Anaerobic biodegradation of aromatic hydrocarbons: pathways and prospects. *J. Mol. Microbiol. Biotechnol.* 15, 93–120. <https://doi.org/10.1159/000121324>.
- Frey, J., Rusche, H., Schink, B., Schleheck, D., 2016. Cloning, functional expression and characterization of a bifunctional 3-hydroxybutanal dehydrogenase/reductase involved in acetone metabolism by *Desulfococcus biacutus*. *BMC Microbiol.* 16, 1–9. <https://doi.org/10.1186/s12866-016-0899-9>.
- Gallacher, C., Thomas, R., Lord, R., Kalin, R.M., Taylor, C., 2017a. Comprehensive database of manufactured gas plant tars. Part C. Heterocyclic and hydroxylated polycyclic aromatic hydrocarbons. *Rapid Commun. Mass Spectrom.* 31, 1250–1260. <https://doi.org/10.1002/rcm.7904>.
- Gallacher, C., Thomas, R., Taylor, C., Lord, R., Kalin, R.M., 2017b. Comprehensive composition of Creosote using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS). *Chemosphere* 178, 34–41. <https://doi.org/10.1016/j.chemosphere.2017.03.050>.
- Ghosh, U., Weber, A.S., Jensen, J.N., Smith, J.R., 2000. Relationship between PCB desorption equilibrium, kinetics, and availability during land biotreatment. *Environ. Sci. Technol.* 34, 2542–2548. <https://doi.org/10.1021/es9905389>.
- Guillén, M.D., Blanco, J., Canga, J.S., Blanco, C.G., 1991. Study of the effectiveness of 27 organic solvents in the extraction of coal tar pitches. *Energy Fuel* 5, 188–192. <https://doi.org/10.1021/e00025a032>.
- Hasegawa, K., Shiojima, S., Koizumi, A., Ikeda, M., 1983. Hippuric acid and o-cresol in the urine of workers exposed to toluene. *Int. Arch. Occup. Environ. Health* 52, 197–208. <https://doi.org/10.1007/BF00526518>.
- Hatheway, A.W., Group, F., 2012a. *Remediation of Former Manufactured Gas Plants and Other Coal-tar Sites, Remediation of Former Manufactured Gas Plants and Other Coal-tar Sites*.
- Hatheway, A.W., Group, F., 2012b. *Remediation of Former Manufactured Gas Plants and Other Coal-Tar Sites. Remediat. Former Manuf. Gas Plants Other Coal-Tar Sites*. CRC press, Taylor & Francis Group, pp. 255–266.
- Hodges, P.W., Richardson, C.A., 1996. EPA method 3550B. Spine (Phila Pa 1976). US-EPA (Environmental Protection Agency).
- Jobelius, C., Ruth, B., Griebler, C., Meckenstock, R.U., Hollender, J., Reineke, A., Frimmel, F.H., Zwienen, C., 2011. Metabolites indicate hot spots of biodegradation and biogeochemical gradients in a high-resolution monitoring well. *Environ. Sci. Technol.* 45, 474–481. <https://doi.org/10.1021/es1030867>.
- Johnston, N., Sadler, R., Shaw, G., Connell, D., 1993. Environmental modification of PAH composition in coal tar containing samples. *Chemosphere* 27, 1151–1158. [https://doi.org/10.1016/0045-6535\(93\)90163-Y](https://doi.org/10.1016/0045-6535(93)90163-Y).
- Jonker, M.T.O., Koelmans, A.A., 2002. Extraction of polycyclic aromatic hydrocarbons from soot and sediment: solvent evaluation and implications for sorption mechanism. *Environ. Sci. Technol.* 36, 4107–4113. <https://doi.org/10.1021/es0103290>.
- Jonker, M.T.O., Smedes, F., 2000. Preferential sorption of planar contaminants in sediments from Lake Ketelmeer, the Netherlands. *Environ. Sci. Technol.* 34, 1620–1626. <https://doi.org/10.1021/es9906251>.
- Kaplan, I.R., Galperin, Y., Lu, S.T., Lee, R.P., 1997. Forensic environmental geochemistry: differentiation of fuel-types, their sources and release time. *Org. Geochem.* 27, 289–317. [https://doi.org/10.1016/S0146-6380\(97\)87941-7](https://doi.org/10.1016/S0146-6380(97)87941-7).
- Kim, D., Yoo, M., Choi, K.Y., Kang, B.S., Kim, T.K., Hong, S.G., Zylstra, G.J., Kim, E., 2011. Differential degradation of bicyclics with aromatic and alicyclic rings by *Rhodococcus* sp. strain DK17. *Appl. Environ. Microbiol.* 77, 8280–8287. <https://doi.org/10.1128/AEM.06359-11>.
- Krauss, M., Singer, H., Hollender, J., 2010. LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. *Anal. Bioanal. Chem.* 397, 943–951. <https://doi.org/10.1007/s00216-010-3608-9>.
- Kueper, B., Wealthall, G., Smith, J., Leharne, S., Lerner, D., 2003. *An Illustrated Handbook of Dense Non-aqueous Phase Liquids (DNAPL) Transport and Fate in the Subsurface*.
- Lee, P.-H., 2000. Use of Solvents for PAHs Extraction and Enhancement of the PAHs Bioremediation in Coal-tar-contaminated Soils.

- Lee, L.S., Rao, P.S.C., Okuda, I., 1992a. Equilibrium partitioning of polycyclic aromatic hydrocarbons from coal tar into water. *Environ. Sci. Technol.* 26, 2110–2115. <https://doi.org/10.1021/es00035a006>.
- Lee, Linda S., Rao, P.S.C., Okuda, I., 1992b. Equilibrium Partitioning of Polycyclic Aromatic Hydrocarbons Form Coal Tar Into Water - f_es00035a006-Lee Article.pdf 26.
- Leibman, K.C., 1975. Metabolism and toxicity of styrene. *Environ. Health Perspect.* 11, 115–119. <https://doi.org/10.2307/3428333>.
- Leutwein, C., Heider, J., 2001. Succinyl-CoA:(R)-benzylsuccinate CoA-transferase: an enzyme of the anaerobic toluene catabolic pathway in denitrifying bacteria. *J. Bacteriol.* 183, 4288–4295. <https://doi.org/10.1128/JB.183.14.4288-4295.2001>.
- Lunge, G., 1909. *Coal-tar and Ammonia*. 4th ed. Gurney and Jackson.
- McGregor, L.a., Gauchotte-Lindsay, C., Nic Daéid, N., Thomas, R., Kalin, R.M., 2012. Multivariate statistical methods for the environmental forensic classification of coal tars from former manufactured gas plants. *Environ. Sci. Technol.* 46, 3744–3752. <https://doi.org/10.1021/es203708w>.
- McRae, C., Sun, C.G., Snape, C.E., Fallick, A.E., Taylor, D., 1999. $\delta^{13}\text{C}$ values of coal-derived PAHs from different processes and their application to source apportionment. *Org. Geochem.* 30, 881–889. [https://doi.org/10.1016/S0146-6380\(99\)00072-8](https://doi.org/10.1016/S0146-6380(99)00072-8).
- Meckenstock, R.U., Annweiler, E., Michaelis, W., Richnow, H.H., Schink, B., 2000. Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. *Appl. Environ. Microbiol.* 66, 2743–2747. <https://doi.org/10.1128/AEM.66.7.2743-2747.2000>. Updated.
- Mohamed, M.E., Seyfried, B., Tschuch, A., Fuchs, G., 1993. Anaerobic oxidation of phenylacetate and 4-hydroxyphenylacetate to benzoyl-coenzyme A and CO_2 in denitrifying *Pseudomonas* sp. *Arch. Microbiol.* 159, 563–573.
- Morasch, B., Richnow, H.H., Vieth, A., Schink, B., Meckenstock, R.U., 2004. Stable isotope fractionation caused by glycol radical enzymes during bacterial degradation of aromatic compounds. *Appl. Environ. Microbiol.* 70, 2935–2940. <https://doi.org/10.1128/AEM.70.5.2935-2940.2004>.
- Ohlenbusch, G., Zwiener, C., Meckenstock, R.U., Frimmel, F.H., 2002. Identification and quantification of polar naphthalene derivatives in contaminated groundwater of a former gas plant site by liquid chromatography-electrospray ionization tandem mass spectrometry. *J. Chromatogr. A* 967, 201–207. [https://doi.org/10.1016/S0021-9673\(02\)00785-9](https://doi.org/10.1016/S0021-9673(02)00785-9).
- Peters, C. a, Luthy, R.G., 1993. Coal-tar dissolution in water-miscible solvents - experimental evaluation. *Environ. Sci. Technol.* 27, 2831–2843. <https://doi.org/10.1021/es00049a025>.
- Richnow, H.H., Annweiler, E., Fritsche, W., Kästner, M., 1999. *Bioavailability of Organic Xenobiotics in the Environment*. Springer, Prague <https://doi.org/10.1007/978-94-015-9235-2>.
- Rixey, W., 1999. Comparison of the fixed-bed and batch leaching characteristics of aromatic compounds in residually trapped crude oils and oily wastes. *J. Hazard. Mater.* 64, 137–156. [https://doi.org/10.1016/S0304-3894\(98\)00231-3](https://doi.org/10.1016/S0304-3894(98)00231-3).
- Safinowski, M., 2005. Anaerobic Biodegradation of Polycyclic Aromatic Hydrocarbons. Safinowski, M., Meckenstock, R.U., 2006. Methylation is the initial reaction in anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. *Environ. Microbiol.* 8, 347–352. <https://doi.org/10.1111/j.1462-2920.2005.00900.x>.
- Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* 48, 2097–2098. <https://doi.org/10.1021/es5002105>.
- Strassman, M., Ceci, L.N., 1963. Enzymatic formation of alpha-isopropylmalic acid, an intermediate in leucine biosynthesis. *J. Biol. Chem.* 238, 2445–2452.
- Thomas, R., Brinckerhoff, P., 2014. *The Manufactured Gas Industry in Europe*.
- Thomas, R., Churchill, S., 2014. *Gas Production Over the Last 200 Years : Friend or Foe ?* pp. 8–15
- Tischler, D., 2015. Microbial styrene degradation. *SpringerBriefs Microbiol* <https://doi.org/10.1007/978-3-319-24862-2>.
- Tischler, D., Kaschabek, S.R., 2012. Microbial styrene degradation: from basics to biotechnology. *Microb. Degrad. Xenobiotics* 67–99.
- Tsai, J.C., Kumar, M., Lin, J.G., 2009. Anaerobic biotransformation of fluorene and phenanthrene by sulfate-reducing bacteria and identification of biotransformation pathway. *J. Hazard. Mater.* 164, 847–855. <https://doi.org/10.1016/j.jhazmat.2008.08.101>.
- Verschueren, K., 2001. *Handbook of Environmental Data on Organic Chemicals*. 1. John Wiley & Sons inc, New York.
- Wanior, J., Ripper, J., 1993. GC, ^{13}C -NMR and IR study of a mixture of waste oil from the old refinery site Pintsch-Oil GmbH i. L. in Hanau, Germany. *Fresenius J. Anal. Chem.* 347, 423–429. <https://doi.org/10.1007/BF00635469>.
- Winter, K., Barton, D., Although, C., 1969. *Thermal Decomposition of Benzoic Acid*.
- Young, L.Y., Phelps, C.D., 2005. Metabolic biomarkers for monitoring in situ anaerobic hydrocarbon degradation. *Environ. Health Perspect.* 113, 62–67. <https://doi.org/10.1289/ehp.6940>.
- Zhang, X., Sullivan, E.R., Young, L.Y., 2000. Evidence for aromatic ring reduction in the biodegradation pathway of carboxylated naphthalene by a sulfate reducing consortium. *Biodegradation* 11, 117–124. <https://doi.org/10.1023/A:1011128109670>.