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Treatment of creosote-contaminated groundwater in a peat/sand permeable barrier—a column study

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Abstract

A column study was conducted to determine if a permeable barrier can be used to treat creosote-contaminated groundwater based on sorption and biodegradation, and to determine which processes remove the various creosote compounds. Creosote-contaminated water (sterile and non-sterile) was applied to sterile and non-sterile saturated columns with peat (20 vol.%) and sand (80 vol.%) for 2 months. Temperature was 9 °C, inlet oxygen concentration 9–10 mg/l and average residence time was two days. The peat/sand barrier material removed 94–100% polycyclic aromatic hydrocarbons (PAHs), 93–98% nitrogen/sulfur/oxygen (NSO)-containing heterocyclic aromatic compounds, and 44–97% total phenols. The peat/sand material efficiently sorbed PAHs (>2 rings) and three-ring NSO-compounds, and also sorbed significant amounts of two-ring NSO-compounds and naphthalene. Naphthalene and NSO-compounds not sorbed were biological degraded. Phenol and cresols were efficiently removed by microbial degradation. The barrier material was somewhat less efficient removing dimethylphenols (DMPs) and trimethylphenols (TMPs), where DMPs were hardly sorbed and TMPs were hardly degraded. The results imply that a peat/sand barrier can treat creosote-contaminated groundwater. Modifications might be needed for enhanced removal of DMPs and TMPs, and oxygen supply might be necessary in aquifers with low oxygen content.

Keywords: Bioremediation; Creosote; Groundwater; Permeable barrier; Sorption

1. Introduction

Permeable reactive barriers are a relatively new in situ technology used to remove pollutants from groundwater. They are installed across the flow path of a contaminant plume, and

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consist of a trench back-filled with barrier material. As the groundwater passes the barrier, pollutants are removed from the water by chemical, physical or biological processes, hence, protecting the surrounding environment. The method offers a simple, cost-efficient way to reduce risk in connection with contaminated sites.

Various materials can be used to remove the contaminants chemically or physically. The most commonly used medium in barriers is zero-valent granular iron [1]. Other barrier materials tested include granular peat moss [2,3], peat [4,5], activated carbon [6], and zeolite [7]. Contaminants tested for removal in barriers include chlorinated solvents [1], gasoline [2,5], BTEX [8], PAHs [6,9], pesticides [7], metals [7,10], arsenite/arsenate [11], uranium [12], nitrobenzene [13] and mine drainage [14].

There is a growing interest in bio-barriers [4,5,8,15–21] where compounds are removed by biodegradation often stimulated by for instance electron donor, nutrients, oxygen or even by addition of microorganisms. If compounds are biological degraded instead of only sorbed, it is possible to extend the lifetime of the barrier and the need for regenerating the barrier material is reduced. Residence time in a barrier is relatively short, from a few hours to several days, depending on groundwater flow, thickness of the barrier, and barrier design (continuous or funnel and gate). In addition, the groundwater temperature is relatively constant, but low (8–12 °C). Hence, it is important that the removal mechanisms can occur relatively fast at low temperatures.

Coal-tar is formed as a by-product in the production of gas from coal, and creosote oil is formed when coal-tar is distilled. Creosote oil has been used for wood preservation for over a century. Spills and sludge deposits on creosote wood preservation sites have led to severe contamination of soil and groundwater. Groundwater leaching from creosote-contaminated sites contains approximately 200 known chemicals [22], consisting of about 17% polycyclic aromatic hydrocarbons (PAHs), 45% phenols and 38% nitrogen/sulfur/oxygen (NSO)-containing heterocyclic aromatic compounds [23]. Due to the potential toxicity, carcinogenicity and mutagenicity of many of these compounds [24], creosote waste sites pose a threat to groundwater resources and to the local environment. Hence, it is important to prevent the spreading of these contaminants.

Most creosote compounds studied in the literature are biological degradable [22] by microorganisms present in groundwater under aerobic conditions [25,26]. Hence, there is a potential for removing them biologically in a permeable barrier where sufficient oxygen is present or supplied. Low molecular PAHs, N- and O-containing heterocyclic compounds, phenol and methylphenols (cresols) can be used as sole carbon and energy sources for a range of microorganisms, while more complex PAHs- and S-containing heterocyclic compounds can probably only be degraded by cometabolism [26,27]. Studies on degradation of dimethylphenols (DMPs) and trimethylphenols (TMPs) in the groundwater environment are limited in the literature. Degradation is often slow and limited, and it is not known if these compounds can be used as the sole carbon and energy source for microorganisms. It has been postulated that microorganisms able to degrade these phenols are not very wide spread in nature [26].

Most of the mentioned studies were conducted with spiked water, pure cultures, or in biofilm reactors, either at high temperatures or long residence times. Hence, they do not reflect the substrate interactions [26,28], natural microbial enrichments and special conditions (residence time, temperature) occurring in a permeable barrier.

All compounds may not be biodegraded during the short period the groundwater is present in the barrier, as degradation can take several days or weeks [25,26]. In addition, the microbial degradation efficiency might be lower during cold periods of the year. Hence, it is important that compounds not degraded are removed non-biologically, by for instance sorption. The creosote compounds vary in hydrophobicity, some are polar and some compounds acts as acids (phenols) and some as bases (N-compounds), hence, it is difficult to find a candidate sorptive medium for the barrier. Nevertheless, at groundwater pH 7–8, the phenols and N-compounds should be present in a non-dissociated form. Sorption of non-ionized organic compounds by soils in aqueous systems is controlled mainly by the organic matter content of the soil [29]. In the barrier, competitive sorption [30] and short residence time can negatively affect sorption efficiency [31]. Activated carbon is known to efficiently remove organic contaminants from water [32], but less costly alternatives are usually preferred. Hence, other materials were tested out for sorption of phenols, where peat was chosen as a good candidate medium [33].

Peat was selected because it is inexpensive and easy to use, and is known to be an efficient barrier material for treating waste water [34–36]. Peat efficiently removes benzene, toluene, *m*-xylene [37] and pentachlorophenol [38], and has also shown removal of phenol [33,39]. Peat is a rather complex soil material with organic matter in various stages of decomposition, containing lignin and cellulose as major constituents. These constituents, especially lignin, contain functional groups, such as alcohols, aldehydes, ketones, acids, phenolic hydroxides and ethers that can be involved in chemical bonding [32]. The specific adsorption for dissolved solids such as polar organic molecules is reported to be high [32]. Peat was mixed with sand to decrease the chance of formation of anaerobic conditions created by microbial decomposition of the organic material, and to optimize hydraulic conditions.

It should be noted that in a sorption/bio-barrier biological degradation is preferred to increase the lifetime of the barrier. Furthermore, transformation of the contaminants does not necessarily require removal and treatment of the barrier material.

The objectives of this research were to determine if a sorption/bio-barrier with peat/sand can be used to treat creosote-contaminated groundwater, and which processes (sorption or biodegradation) remove the various compounds.

2. Materials and methods

2.1. Experimental set-up

Six stainless steel columns (40 cm high × 10 cm in diameter) were packed with 25 cm barrier material (80% sand/20% peat) and 8 cm fine sand (Fig. 1). Three columns were kept sterile to monitor sorption and three were biologically active to monitor biodegradation. The columns were closed in both ends with stainless steel caps. Before the experiment started, the non-sterile columns were flushed with 7 l of purified water, and sterile columns were flushed with 7 l of purified water containing sodium azide (0.5 g NaN_3/l). Contaminated water was filled into six Tedlar® bags (Sigma-Aldrich), and transferred to each column in upflow mode. The water was driven by a peristaltic pump (Gilson Medic with Watson Marlow Marprene tubes), at a rate of 600 ml per day. The effective pore-volume in the

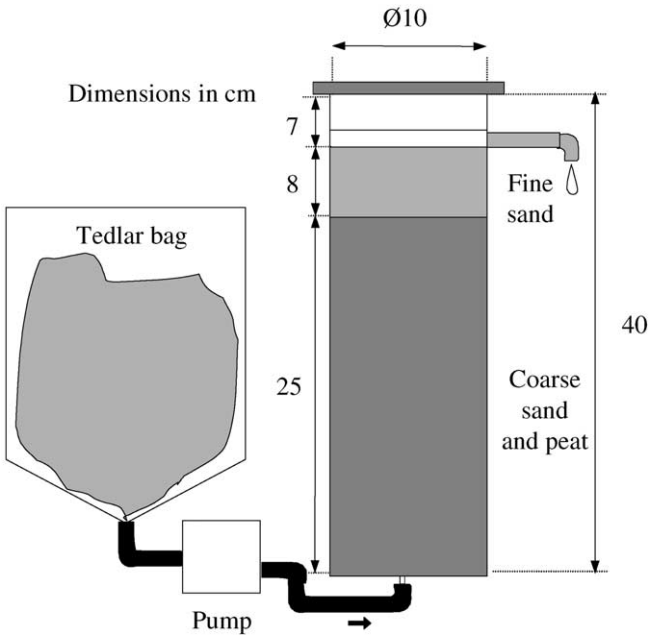

Fig. 1. Experimental set-up.

columns was approximately 220 ml, and the total pore-volume was approximately 1130 ml. A piece of fiber glass cloth was placed over the inlet in the base cap to prevent loss of barrier material. The water was allowed to flow out through a tap just above the barrier material, 33 cm from the bottom of the column. Black ISO-Versinic tubes were used for the external connections between the columns, pump tubes and inlet water. The experiment was conducted at 9 °C, which is the average groundwater temperature at the site where the groundwater was collected.

Thirty milliliters samples were collected every two to three days in 60 ml amber glass bottles. The bottles were capped with plastic caps (not in contact with sample) and kept frozen until analyzed. Samples collected for microbial analysis were analyzed immediately after sampling. Oxygen was measured directly in the water phase above the barrier material.

2.2. Residence time

Residence time of the water in the columns was determined in the beginning and end of the experiment. Before the experiment started, the columns were filled with purified water. The contaminated water had a higher electrical conductivity than purified water, hence, its breakthrough in the column outlet could be followed by measuring conductivity in the effluent water (once a day). At the termination of the experiment, the residence time was determined by pumping a chloride tracer (sodium chloride in water, conductivity of 3.7 mS/cm) through the column, and measuring conductivity of effluent samples until breakthrough.

Table 1
Chemical and physical characteristics of materials used in the columns

Parameter	Peat	Sand	Top sand	Sand/peat
pH	4.1	9.1	8.3	5.3
Electrical conductivity (μS/cm)	46	49	28	[a]
Cation-exchange capacity (meq/100 g)	120	3.4	1.9	[a]
Volume weight (kg/l)	0.06	1.69	1.74	[a]
Organic carbon (g/100 g)	42	0.1	<0.1	[a]
Nitrogen (g/100 g)	0.69	<0.05	<0.05	[a]
Ammonium (mg/kg)	263	<0.5	<0.4	[a]
Nitrate + nitrite (mg/kg)	15	<0.5	3.6	[a]
Phosphorus (mg/kg)	193	323	404	[a]
o-Phosphate (mg/kg)	36	0.62	0.24	[a]

[a] Not analyzed

2.3. Barrier material

The characteristics of the components of the barrier materials are listed in Table 1, and the grain size distribution in the two sands is presented in Table 2. Note that 12 and 4.6% gravel (>2 mm) were removed from the barrier sand and top sand, respectively, before grain size analyses. A Sphagnum peat (Kallak Peat-Industry, Trogstad, Norway) was sieved through a 6 mm sieve to remove large moss pieces. The peat was saturated with purified water. The barrier material was prepared by mixing 500 ml peat saturated with water (40 g dry weight (DW)), 1900 ml coarse sand (2740 g DW), and 4 g crushed granulated fertilizer (NPK14-3-18). The barrier material contained 1.44% organic material by weight, and 0.61% organic carbon. On top, 700 ml fine sand (850 g DW) was added to keep the barrier material in place. The barrier material used in the abiotic columns was sterilized using 32 kGy gamma radiation.

2.4. Influent water

Creosote-contaminated groundwater was collected from groundwater wells at a closed-down wood preservation site in Hommelvik, near Trondheim, Norway. A detailed site description is given in Sparrevik and Breedveld [40]. The water was mixed and stored in 25 l plastic containers at −20 °C until used. Before use, the water was thawed at 9 °C for 3–4 days. The contents of four containers were mixed in a 200 l plastic barrel, and distributed

Table 2
Grain size distribution in sand samples

	Sand: grain size (mm)			Silt: grain size (mm)			Clay: grain size (<0.002 mm)
	2–0.6	0.6–0.2	0.2–0.06	0.06–0.02	0.02–0.006	0.006–0.002	
In barrier material (wt.%)	36.1	46.4	13.7	1.4	0.8	0.0	0.16
Top sand (wt.%)	18.4	55.4	22.6	1.2	0.8	0.0	0.16

back into the containers. Equal amounts (15 l) of water were pumped into six 25 l collapsible Tedlar® bags, where one bag was connected to the inlet of each column. The water being used for the three sterile columns were added sodium azide (0.5 g/l) to inhibit bacterial activity, immediately before pumped into the Tedlar® bags. The bags were refilled every 2 weeks.

2.5. Analytical methods

To analyze for creosote components in water, 10 ml of the aqueous sample was poured into a 16 ml Kimax glass cylinder and capped with a Teflon lined screw cap. A blank and control were prepared, and internal standard (acenaphtene-d10, phenanhtrene-d10, chrysene-d12, perylene-d12 and 2-bromophenol) was added to all samples. The phenols in the samples were derivatized prior to the extraction with methylchloroformate in basic solution (pH 8–9 adjusted by concentrated HCl and borax) by using the method described by Grahl-Nielsen and Landgren-Skjellerudsveen [41]. The sample was extracted twice with dichloromethane (1 and 0.5 ml) by shaking for 2 min. The solvent was transferred to a Kimble test tube using a Pasteur pipette. Excess water was removed with anhydrous sodium sulfate. The extract was transferred to 2 ml GC vials, capped and analyzed on a GC/MS (HP6890GC with a 5976 mass selective detector). The GC was equipped with a 30 m × 0.25 m i.d. WCOT fused silica capillary column (Chrompack, CP-SIL 8 CB low bleed). Helium at 15 psi was used as carrier gas. The injector temperature was held at 320 °C. The GC oven temperature was held at 60 °C for 1 min, increased to 160 °C at 25 °C/min, held for 5 min, then increased from 160 to 320 °C at 10 °C/min. An internal standard calibration method was used.

Standard solutions were prepared from a stock standard mixture of 16 PAHs (USEPA) and 14 phenols diluted to four concentration levels in methanol. Likewise a mixture of five NSO-compounds was used to prepare three calibration standard concentrations for their determination. A volume of 1 ml of each standard solution of each concentration was added to 9 ml purified water. The standard samples were treated identically as the samples. Fourteen phenols were analyzed, included phenol, three methylphenols (*o*-, *m*-, *p*-cresol), six DMPs (2,6-, 2,5-, 2,4-, 3,5-, 2,3-, 2,3-, 3,4-DMP) and four TMPs (2,4,6-, 2,3,6-, 2,3,5-, 3,4,5-TMP). Three two-ring NSO-compounds were analyzed (benzofuran, benzothiophene, quinoline), as well as two three-ring N-and O-containing compounds (dibenzofuran and carbazole) and 16 PAHs (2–6 rings).

Conductivity was measured using a Wissenschaftlich Technische Werkstatten 90 conductivity analyzer with temperature correction, and oxygen was measured using an OXI 96 WTW Microprocessor OXI meter.

pH in the barrier material was measured in a solution with 10 g material (wet weight) and 25 ml purified water. The mixture was shaken for 2 h, and allowed to rest 10 min before measuring pH in the supernatant by using an ion-selective electrode (Orion Ross, Hanna Instruments HI 931402 Microprocessor pH meter).

2.6. Microbial numbers

Number of creosote degraders in barrier material and influent and effluent water samples, and number of viable microorganisms in the barrier material, were determined by

a most probable number (MPN) method. For enumeration of microbial populations in barrier material, aqueous extracts of 10 g material were prepared using 90 ml sterile pyrophosphate (0.05 M $Na_2P_2O_7$) (pH 8) in 100 ml glass bottles. The bottles were shaken for 30 min at 250 rpm, and particles were allowed to settle for 5 min before a 200 μl liquid sample was taken out for analysis. For analysis of liquid samples, 200 μl liquid samples were taken directly out for analysis. The samples were serially diluted in sterile 24-well Corning microtiter plates containing 1.8 ml 5 g/l Tryptic Soy Broth. The inoculated plates were incubated at 15 °C for 14 days. Wells were scored as positive when the liquid was turbid.

The number of viable creosote-degrading organisms was enumerated as mentioned above by inoculating in Bacto Bushnell-Haas Broth (3.27 g/l), and adding one drop (10–20 μl) creosote to each well. The plates were incubated for 21 days. To determine positive wells, a 20 μl fluorescein diacetate (FDA) solution (2 g FDA/l acetone) was added to all wells. Within 1–2 days, enzymes in positive wells hydrolyzed FDA to fluorescein, which is observed by its green color.

The total number of bacteria in influent and effluent water samples was enumerated by epifluorescence microscopy with SYBR green staining, as described by Noble and Fuhrman [42]. For each filter, at least 30 fields were counted on a Leica DMRBE with 100× objectives under blue excitation light (I3 filter cube, excitation filter BP 450–490, suppression filter LP 515). Bacteria cells were enumerated on two filters per sample.

3. Results

3.1. Residence time

The residence time in the columns was determined in the beginning and in the end of the experiment, by measuring conductivity in outlet water samples. Fig. 2 shows how the conductivity changed as purified water was replaced by contaminated water in the beginning of the experiment. The same result was obtained by using chloride as a tracer after the termination of the experiment. The mean residence time was found to be 50 h in both cases.

3.2. Changes in inlet water concentrations over time

During the experimental period we observed that several of the creosote compounds were significantly degraded in the non-sterile inlet water during the 2–3 weeks period between refilling the Tedlar® bags. Hence, the inlet concentrations in the sterile and non-sterile columns were only identical on the days of filling and refilling the bags. The changing concentrations did not seem to significantly influence the results. However, we felt that in order to compare the treatment processes in the barrier material it was most correct to sample the columns three days after refilling the Tedlar® bags, when the new inlet water had completely passed through the barrier material (Fig. 2). In the beginning of the study though, it was important to observe when the different compounds started escaping the columns. Therefore, we included several data point during the first 18 days in the results presented here (after the Tedlar® bags were filled the first time).

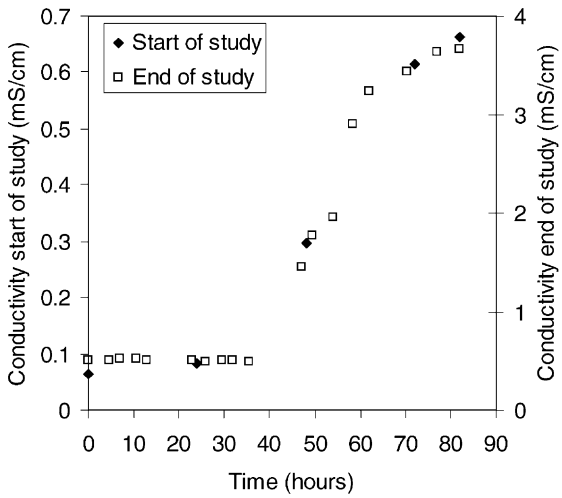

Fig. 2. Change in conductivity measured in the outlet of the biological active column in the beginning of the experiment when contaminated water was added and after the termination of the experiment when a chloride tracer (NaCl) was added.

3.3. Removal in columns

Table 3 shows inlet and outlet concentrations (average of the three last data points shown in Figs. 3–5) and the total percentage of the compounds removed in non-sterile columns. In addition, it shows the distribution of the total percent removal between non-biological processes (sterile columns) and biological processes (difference between total percent removed and removal by non-biological processes). The grouping of the compounds were explained in analytical methods. It should be noted that the 16 PAHs were divided into naphthalene (two-ring PAH) and 15 PAHs (3–6 rings).

The PAHs were dominated by naphthalene in the creosote-contaminated inlet water (Table 3). The 15 PAHs were dominated by three- and four-ring compounds, and the

Table 3
Average inlet and outlet concentrations and removal efficiency in columns

Compounds	Concentration ± S.D. (μg/l)			Total	Removal efficiency (%)	
	In	Out, sterile	Out, non-sterile		Non-biological	Biological
Phenol + cresols	320 ± 8	319 ± 54	10 ± 3	97	0	97
Total DMPs	1252 ± 53	1141 ± 87	436 ± 166	65	9	56
Total TMPs	508 ± 93	325 ± 30	285 ± 69	44	36	8
Naphthalene	893 ± 141	340 ± 68	3 ± 1	100	62	38
Sum 15 PAHs	170 ± 27	17 ± 7	10 ± 1.8	94	90	4
Two-ring NSOs	647 ± 94	228 ± 99	44 ± 16	93	65	28
Three-ring NSOs	93 ± 14	6 ± 5.5	2 ± 1	98	94	4

The concentrations are average values of the three last data-points presented in Figs. 3–5.

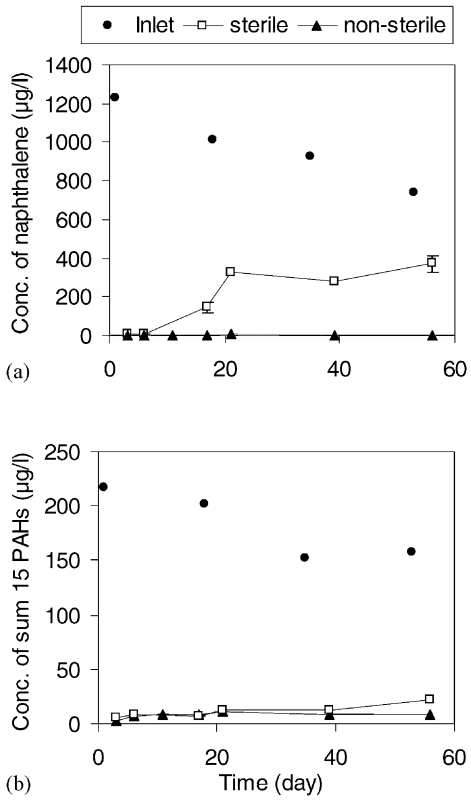

Fig. 3. Concentration of (a) naphthalene and (b) sum 15 PAHs in creosote-contaminated groundwater in inlet water, and after treatment in sterile and non-sterile columns. Error bars indicate minimum and maximum concentrations where two or three columns were sampled.

five- and six-ring compounds were only present in less than 1 μg/l (not shown in the table). The barrier material efficiently removed naphthalene and the other 15 PAHs (Table 3 and Fig. 3). The major removal processes for PAHs were non-biological, although 38% of the naphthalene was degraded biologically. Concentrations in the non-sterile column effluents varied from <0.01 (five- and six-ring PAHs) to 4.9 μg/l (anthracene).

Among the phenols, DMPs dominated in the inlet water, followed by TMPs (Table 3). Phenol and cresols were efficiently removed in the biological active barrier material, whereas the treatment efficiency was lower for DMPs and TMPs (Table 3 and Fig. 4). In general, all removal of phenol and cresols was biological, the main removal of DMPs was biological, whereas the main removal of TMPs was non-biological except from the last day of sampling. The biological treatment efficiency seemed to improve somewhat over time for DMPs and TMPs, and might indicate adaptation of the microorganisms (Fig. 4b and c). After 4 days only, the outlet concentrations of phenol, cresols and DMPs were identical as the inlet water concentrations (Fig. 4a and b). Some of the TMPs sorbed to the barrier material hence, the concentrations in the outlet were never the same as the inlet concentrations during the 2

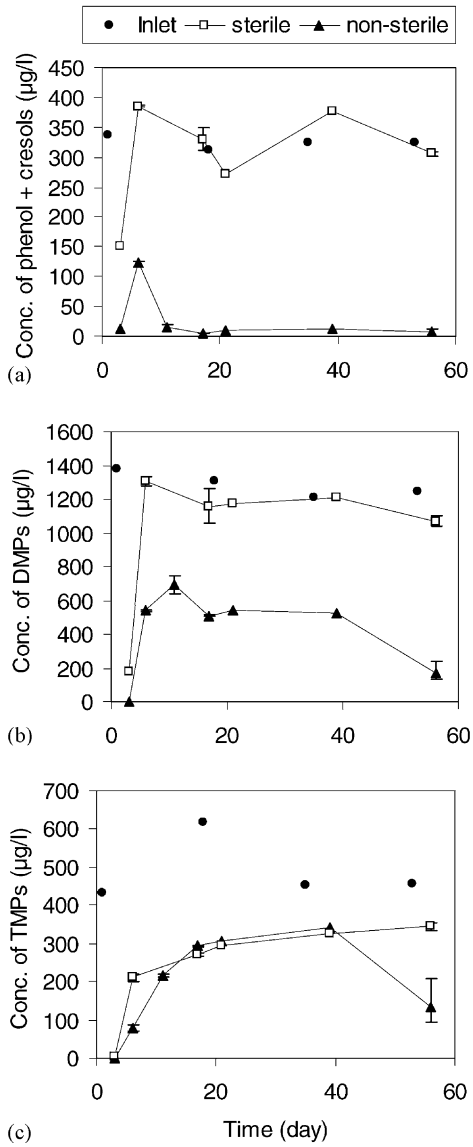

Fig. 4. Concentration of (a) sum phenol and cresols, (b) sum DMPs and (c) sum TMPs in creosote-contaminated groundwater in inlet water, and after treatment in sterile and non-sterile columns. Error bars indicate minimum and maximum concentrations where two or three columns were sampled.

months experiment (Fig. 4c). Concentrations in the non-sterile column outlets varied from 0.2 (*m*-cresol) to 258 μg/l (2,3-DMP) during the study.

In the inlet water, two-ring heterocyclic compounds, in particular quinoline and benzothiophene (65 and 19%, respectively) dominated the analyzed NSO-compounds. The

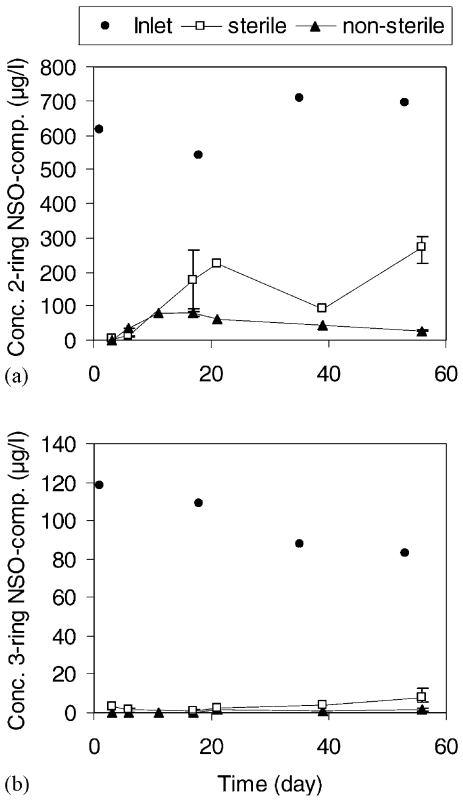

Fig. 5. Concentration of (a) three two-ring NSO-compounds and (b) two three-ring NSO-compounds in creosote-contaminated groundwater in inlet water, and after treatment in sterile and non-sterile columns. Error bars indicate minimum and maximum concentrations where two or three columns were sampled.

combined effect of sorption and microbial degradation in the non-sterile barrier material resulted in efficient removal of all two-ring NSO-compounds (93% in average, Table 3). The trend in Fig. 5a indicates that the removal efficiency improved somewhat over time. Although the main removal process was non-biological, significant amounts of the compounds were also removed biologically. Quinoline sorbed best of the two-ring compounds (69%), and outlet concentrations varied from 4 to 30 μg/l. Benzothiophene was the most difficult NSO-compound to remove, and outlet concentrations ranged from 20 to 65 μg/l. The three-ring heterocyclic hydrocarbons were efficiently removed in the barrier material during the 2 months study, mainly by sorption (Table 3 and Fig. 5b). The concentrations were below 4 μg/l in the outlet water.

The degradation that occurred in the Tedlar® bags indicates that significant amounts of creosote compounds can quickly be removed from groundwater at 9 °C due to aerobic microbial degradation, although no barrier material was added. In order to compare the removal of creosote compounds occurring in the barrier material with removal occurring in the water in the same time period, concentration changes in the water during a three day

Table 4
Disappearance of creosote components in water contained in a Tedlar® bag during 3 days at 9 °C

Compounds	Start (μg/l)	After 3 days (μg/l)	Removal efficiency (%)
Phenol + cresols	239	224	6
Total DMPs	1241	1308	0
Total TMPs	458	506	0
Naphthalene	738	5	99
Sum 15 PAHs	315	116	63
Two-ring NSOs	696	491	29
Three-ring NSOs	83	62	25

period is presented in Table 4. Analysis of sterile water from Tedlar® bags showed that the removal was mainly due to biological processes (not shown). Although the removal efficiency in the water could not compare with the removal occurring in the barrier material (i.e. no phenols were removed), significant amounts of PAHs and NSO-compounds were degraded in the water contained in Tedlar® bags. According to Table 3, the PAHs and three-ring NSO-compounds were sorbed to the barrier material, and not removed by biological processes, whereas the results in Table 4 show that these compounds were significantly biodegraded when not sorbed to the barrier material (Table 4). These results imply that even though the main removal processes apparently is sorption, the compounds will be biological degraded in the columns, if they are bioavailable or released from the barrier material.

3.4. Number of microorganisms

Fig. 6 shows the number of total bacteria (SYBR) and viable creosote degraders in water from column effluents throughout the study. The figure shows that the number of

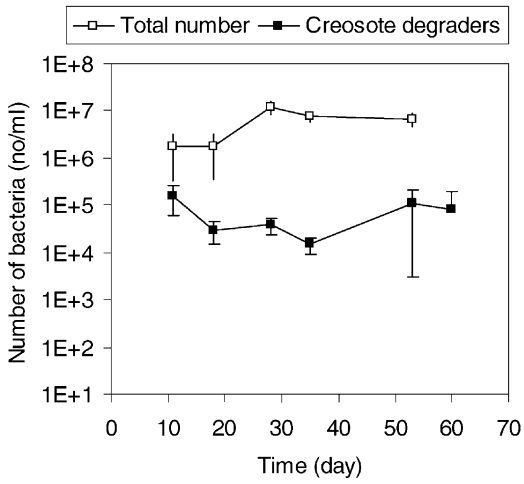

Fig. 6. Number of total bacteria (SYBR) and viable creosote degraders (MPN) in creosote-contaminated groundwater in effluent water throughout the study (average of three columns). Logarithmic scale, error bars show S.D.

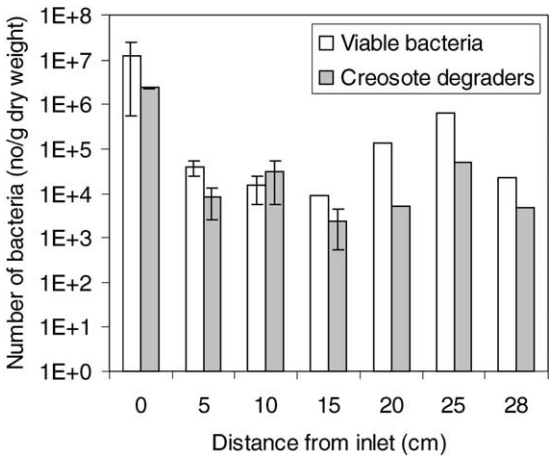

Fig. 7. Number of total viable bacteria (MPN) and viable creosote degraders (MPN) in barrier material at different distances from the column inlet. Logarithmic scale, error bars indicate minimum and maximum values.

bacteria were fairly stable through the 2 months study. The number of creosote degrading organisms in the inlet water ranged from 4.65×10^2 to 1.15×10^4 ml^{-1} when the thawed creosote-contaminated water was added to the columns. After the water had been in the Tedlar® bags for about 2 weeks, the number of viable creosote degraders ranged from 3.75×10^4 to 3.75×10^5 ml^{-1}. These numbers were similar to the numbers found in column outlets.

Purified water was flushed through the columns before the experiment started. At this point the number of creosote degrading organisms was 115–215 ml^{-1} in the effluent water, and the number of total bacteria ranged from 4.6×10^6 to 7.0×10^6 ml^{-1}.

At the end of the experiment, samples of barrier material were collected from various levels in one of the columns, and the number of viable bacteria (measured by MPN) and viable creosote degraders were determined (Fig. 7). The numbers of bacteria were highest in the inlet (0 cm). There was no clear trend in the rest of the column.

3.5. Physical and chemical water characteristics

Table 5 shows chemical and physical characteristics of the water measured in inlet water and outlet water of sterile and non-sterile columns. The effluent was analyzed after three days when the contaminated water arrived at the outlet, and at the end of the experiment (day 60).

The pH of the barrier material was 5.3 at the start of the experiment, and at the end was measured as 7.4–7.7 and 7.9–8.0 in non-sterile and sterile barrier material, respectively (measured at five different points in the column). Sodium azide used to sterilize the water lead to increased sodium concentrations, increased conductivity and somewhat elevated pH levels. The base quinoline has a pK_a (dissociation constant) of 4.9, and the phenols have a pK_a of >9.9 [43], hence, the compounds should be present in non-dissociated form at pH between 6.9 and 8.4 [44]. pH differences in sterile and non-sterile columns should, thus, not lead to differences in sorption processes. The high nutrient values in the outlet

Table 5
Chemical and physical characterization of inlet water and outlet water from sterile and non-sterile columns at the start (day 4) and end (day 60) of the experiment

Parameter	Inlet water		Outlet: start		Outlet: end	
	Non-sterile	Sterile[a]	Non-sterile	Sterile	Non-sterile	Sterile
pH	7.0–7.9	7.0–8.1	7.1–7.7	7.8–7.9	7.5–7.5	8.1–8.7
Conductivity (mS/cm)	0.5–0.6	1.3	0.6	1.4–1.5	0.6	1.3
Oxygen (mg/l)	8.8–10.3		2.3–5.7[b]	9.0–9.6[b]	2.3–5.7[b]	9.0–9.6[b]
Iron (total; mg/l)	1.9		0.23	0.56	1.05	2.19
Magnesium (mg/l)	0.8		0.67	0.38	4.04	1.63
Sodium (mg/l)	17		64	218	18	192
Sulfate (mg/l)	29		28	28	29	29
o-Phosphate (mg/l)	0.029		1.14	0.58	0.04	0.61
Nitrate + nitrite (mg/l)	0.025		0.015	0.036	0.0072	0.0054
Ammonium (mg/l)	0.96		11	17	0.6	6.1
Total organic carbon (mg/l)	37		32	24	27	36

[a] Same concentrations as in the non-sterile columns, except from factors influenced by the addition of NaN_3. Sodium was not measured.
[b] Concentration range measured through the whole period.

of all columns in the beginning of the study were due to the added granulated fertilizer in the barrier material. Some of the nutrients were washed out throughout the experiment, and the microorganisms seemed to have used all of the supply within the end of the study. Nevertheless, this had no influence on the microbial numbers or efficiency of degradation, indicating that there was sufficient supply of nutrients in the water or barrier material.

3.6. Oxygen

The oxygen level in the groundwater added to the Tedlar® bags varied between 8.8 and 10.3 mg/l (Table 5). To measure oxygen in the column outlets, the oxygen probe was placed in the 0.5 cm water layer that had formed above the barrier material. This water was in contact with air, hence, the concentrations in the barrier material are expected to be somewhat lower than measured. The highest concentrations were usually measured three days after the Tedlar® bags were refilled, when samples for chemical analysis were collected. After 2 weeks in the Tedlar® bags, the oxygen concentration had not changed in sterile water, but had decreased to 2.2–4 mg/l in non-sterile water. There was no aerobic microbial activity in the sterile columns, as indicated by the stable oxygen levels in the water that had passed through the columns (Table 5).

4. Discussion

4.1. Treatment in peat/sand barrier material

The purpose of this study was to determine if a sorption/bio-barrier can be used to treat creosote-contaminated groundwater, to determine if peat/sand is an effective barrier material, and to determine which processes remove the various compounds.

The results show that creosote-contaminated groundwater can be efficiently treated for PAHs, NSO-compounds, phenol and cresols in a peat/sand barrier material, at low temperature (9 °C), in a system that simulates the short residence time in a barrier.

Peat efficiently sorbed PAHs (>2 rings) and three-ring NSO-compounds, and also sorbed significant amounts of two-ring NSO-compounds and naphthalene. The NSO-compounds and naphthalene not sorbed were biological degraded. The microbial community in the barrier material also efficiently degraded phenol and cresols. DMPs were hardly sorbed, but 50% were biological degraded. A portion of the TMPs were sorbed, but they were hardly degraded.

The results imply that sorption/bio-barriers can be used to treat creosote-contaminated groundwater, as well as other organic contaminants. The method might need improvement for removing DMPs and TMPs. Although the removal of DMPs and TMPs was less efficient, it might meet local treatment criteria, depending on use of the water. 2,4,6-TMP was found to be the most toxic compound of selected creosote compounds, for freshwater flea *Daphnia magna* (48 h LC50 = 300 μg/l) [45], while it is not very toxic towards the marine bacteria *Vibrio fischeri* (Microtox®, EC50 = 11,900 μg/l) [46]. Using Microtox®, the toxicity was somewhat higher for the other TMPs, similar for the DMPs and lower for phenol [46]. Hence, the criteria for treatment efficiency will depend on recipient and water use, and the method might need to be improved by changing conditions or type of barrier material.

Sufficient amounts of oxygen are required to achieve efficient biological degradation of creosote compounds [26,47] in the permeable barrier although slow anaerobic degradation might occur [25,26,48–50]. The groundwater used in this study contained 8.8–10.3 mg/l oxygen, which is more than expected to be present in an aquifer. Oxygen might need to be supplied in a full-scale barrier, for instance by biosparging, airsparging or use of oxygen release compounds (ORC®) [8,16–19]. Addition of oxygen can lead to volatilization of some contaminants, or decrease hydraulic conductivity because of iron precipitation or biofouling [6,8].

4.1.1. Treatment of phenols

The results observed for phenols were in agreement with other studies conducted in batch, columns or biofilm reactors, showing efficient degradation of phenol and cresols [51–53] and slow degradation of DMPs and TMPs [52,54,55]. 2,4,6-TMP was not degraded at all (data not presented), which was also observed by Arvin et al. [52]. Although Mueller et al. [51] have shown that all DMPs are rapidly degraded in a slurry phase at 28.5 °C, it might not be possible to obtain the same results in the groundwater environment. Several authors have observed that when methylated phenols are present in a mixture, there is a preferential degradation of phenol and cresol [25,52,53]. Arvin et al. [52] suggested that there was an inhibition of the degradation of the more slowly degradable phenols. If this is the case, degradation should improve when the least methylated phenols are removed. Hence, the removal might improve with a longer residence time in the barrier. Alternatively, the DMPs and TMPs might be remediated by natural attenuation in the zone following the barrier. Dyreborg [26] postulates that microorganisms degrading DMPs are more rare than microorganisms degrading PAHs and other phenolic compounds, indicating that biodegradation might be difficult. Furthermore, it is unclear whether disappearance of phenol and cresols decrease or increase degradation of DMPs [53].

It might also be difficult to remove phenols by sorption, because they are polar and very water-soluble. Nevertheless, phenol, *o*-cresol and 2,4-DMP seems to sorb onto peat in batch experiments [33,39]. It was also expected that other DMPs and TMPs would sorb at least as well or better because of a higher degree of methylation [56] and higher octanol–water partitioning coefficient (K_{ow}). The results from the column study where only 36% of the TMPs sorbed to peat were, thus, not anticipated, although it is known that sorption coefficients are usually higher when measured in batch than in column studies. Reasons why sorption of all phenols was low in the columns might be due to short residence time so that equilibrium was not reached, or competition with other compounds. NSO-compounds are known to inhibit sorption of each other [30], and might also inhibit sorption of other creosote compounds. Furthermore, sorption of some phenols can also be adversely affected by the presence of other phenolic compounds [57], where the competitive effect is more pronounced with the increasing number of compounds in solution [56]. If PAHs and NSO-compounds are rapidly removed from solution there might be less competition and increased sorption of phenols. Using more organic material, or a more matured peat that is less polar and more aromatic, might also improve sorption of non-dissociated phenols [58].

4.1.2. Treatment of PAHs

Sorption processes dominated the removal of all PAHs, including naphthalene. This was also expected, as PAHs are known to sorb to organic matter [59]. Naphthalene did not sorb as well as the other PAHs. It was present in higher concentrations and has a higher water solubility and a lower K_{ow}, resulting in lesser sorption than the other PAHs [59]. It should also be noted that the residence time in the columns might not be sufficient to obtain equilibrium, which might take a few hours to several days [31,60].

The PAHs might sorb to the peat before they are biological degraded, and thus, made unavailable for the microorganisms in the barrier material. In that case, sorption sites in the barrier might be filling up over time. However, significant amounts might be degraded before the compounds are sorbed in inaccessible sorption sites. Scow and Johnson [61] present in a review that sorption of non-polar organic compounds in organic matter generally occur in two stages. Roughly 1–50% of the chemical will quickly enter a labile sorbed phase (minutes to hours) with the remaining chemical slowly entering a resistant sorbed phase (days to months). Furthermore, the review shows that some bacteria strains are able to degrade compounds in the labile phase (shown for naphthalene), whereas chemicals in the resistant sorbed phase are regarded unavailable for microorganisms. The compounds will be available again when desorbed.

The microorganisms are probably able to degrade much of the PAHs present in the barrier material, but the degradation would probably be even larger if the PAHs did not sorb to organic material. This was indicated by the results in the Tedlar® bags, where the PAHs present in the water were significantly degraded within three days. Hence, there is a potential for extending the longevity of the barrier if the PAHs are kept available in a phase of the barrier where no organic matter is present. This is possible if two different barrier materials are installed in series. The first zone of the barrier might consist of sand (no organic matter for sorption) where the hydrophobic compounds are degraded but not sorbed. Sand with a high surface area should be an efficient support material for microorganisms [62]. A second zone with organic material (peat and sand) is necessary for sorption and further

degradation of compounds not sufficiently removed during the first zone (e.g. phenols). If most compounds are degraded in the first zone, there might also be less competition for sorption sites in the second zone.

4.1.3. Treatment of NSO-compounds

The three-ring heterocyclic-compounds sorbed very well, and even the less hydrophobic two-ring NSO-compounds sorbed to some degree. Quinoline sorbed better than both of the other two-ring NSO-compounds and naphthalene, although this compounds has the lowest K_{ow} and highest water solubility of all the analyzed heterocyclic compounds. Quinoline is a base (pK_a 4.9), but expected to be present mainly in non-dissociated form at pH above 6.9 [44], which was the case in this study. Nevertheless, Bailey et al. [63] found that adsorption of basic compounds seems to be principally dependent upon the surface acidity and not upon the pH of the bulk solution. Hence, the quinoline could be present as a cation at peat surfaces where pH is low, and thus, be more strongly sorbed [64,65]. Using a different organic material with high pH might, therefore, not show the same sorption abilities for quinoline.

When present in the Tedlar® bags all NSO-compounds were significantly degraded within three days, showing that the compounds are easily degradable when not sorbed. Hence, a two-zone system as mentioned for the PAHs might be an advantage for the lifetime of the barrier.

The removal efficiency of NSO-compounds improved over time, which might indicate that the NSO degraders grew more slowly, or needed to be adapted to the compounds. Hence, a barrier might not work efficiently for removal of NSO-compounds initially.

4.2. Interactions

The observed degradation in the Tedlar® bags shows how easily the compounds can be degraded biologically when oxygen is present, although no additional nutrients were added, at low temperature (9 °C), and even when present in a complex mixture. This is in good agreement with the study of Arvin et al. [66] documenting that free-living groundwater bacteria are capable of extensive degradation of aromatic hydrocarbons and NSO-compounds. Nevertheless, the degradation of phenols was delayed in the water (Tedlar® bag) compared to in the barrier material. Naphthalene or NSO-compounds might have affected the degradation. However, no negative substrate interaction was reported between naphthalene and phenols in studies cited by Dyreborg [26]. NSO-compounds, on the other hand, are known to have a negative effect on the degradation of other creosote compounds, and are believed to act as toxicants on some creosote degraders [67–69]. The presence of these compounds in the water might, thus, inhibit degradation of phenols. The barrier material might have prevented these negative effects by removing naphthalene and NSO-compounds from solution by sorption.

Benzothiophene is only known to be degraded by cometabolism, where the compound cannot be used as a growth substrate, but is degraded when microorganisms use a different substrate as a carbon and energy source [68,70]. The degradation of benzofuran is probably also stimulated by the presence of other substrates [70]. The presence and degradation of naphthalene, and possible other creosote compounds, might be crucial for the degradation of benzothiophene and benzofuran [26]. Hence, a two-zone barrier where all naphthalene

is available for microbial degradation, and not sorbed, might also further stimulate the degradation of benzothiophene and benzofuran.

4.3. Microorganisms

Creosote degrading organisms were present in the barrier material and in the inlet groundwater when the experiment started. They multiplied about 10-fold both in the inlet and outlet water. Microorganisms able to degrade creosote compounds are known to be widely spread in nature [26], hence, it was not surprising that they are present in peat which is composed of complex phenolic and complex aromatic compounds. Cresols are for instance used by microbial isolates from uncontaminated sites, because the compounds resemble the monomeric components of the humic and fulvic acids that likely comprise the bulk of the naturally available organic matter in the aquifer [71]. The fact that these microorganisms were already present might have improved degradation of phenols, led to efficient degradation of all compounds from the beginning of the study, and prevented or reduced toxicity effects caused by the creosote contaminants.

It should be noted that only the inlet and outlet water were sampled throughout the study, and it is assumed, that the microbial numbers in the outlet reflect trends in the microbial population (not numbers) in the column.

The microbial population seemed to have adapted rapidly to contaminant load, and there was no obvious lag time before degradation started in the columns. Hence, if degrading microorganisms are present in the contaminated groundwater, it seems like a biologically active barrier can be efficient immediately. As long as the barrier is fed contaminated water, it seems that the number of degrading organisms is relatively stable.

The effect of nutrient addition may or may not have any effect on degradation of creosote-contaminants [72]. Inorganic nutrients were added in the barrier material in our study, but it appeared that it might not have been necessary. The addition of peat might keep the barrier self sufficient with nutrients [2]. The number of creosote degrading organisms was about the same in the inlet water as in the outlet water when counted after 14 days, although no nutrients were supplied to the inlet water. It should be noted that the water was collected from an area that might have been supplied with nitrogen and phosphorus from an in situ biosparging experiment [40]. Although the concentrations of inorganic nutrients declined in the columns over time, the number of creosote degrading organisms stayed intact.

Creosote degraders were present through the whole column, but they were concentrated in the inlet. Only five cm from the inlet, the number of creosote degraders had decreased significantly. Oxygen concentrations might be one reason for these patterns, as the concentrations are highest in the inlet. This is in agreement with results found by Barbaro et al. [73], where the distribution of cultivable microorganisms at a contaminated site (Borden, Canada) was related to depth and dissolved oxygen levels. Furthermore, the substrate was both more abundant and probably more easily degradable in the inlet of the columns. Hence, only the more recalcitrant compounds were available throughout the columns. Based on the numbers and distribution of creosote-degraders in the barrier material, it seemed like most of the creosote compounds are degraded in the first centimeters of the barrier material.

If the microbial numbers will be equally high in the barrier, it means that the zone for microbial degradation does not have to be very thick. This hypothesis needs to be confirmed

by experiment. It should be noted that much of the creosote degraders might also be present in the groundwater as observed by Arvin et al. [66], and also indicated in this study, hence, degradation might also occur in the water by the free-living organisms while it flows through the column.

5. Conclusions

This study implies that a sorption/bio-barrier can be used to treat creosote-contaminated groundwater. Creosote-contaminated groundwater was efficiently treated for PAHs, NSO-compounds, phenol and cresols, using a peat/sand barrier material under aerobic conditions, at low temperature (9 °C) and short residence time (2–3 days). Peat mixed with sand (20/80 vol.%) lead to efficient sorption of PAHs, three-ring heterocyclic compounds and also significant amounts of naphthalene and two-ring NSO-compounds. Phenol and cresols were readily degraded, in addition to naphthalene and two-ring NSO-compounds. Although significant amounts of DMPs were degraded, optimization of the method might be necessary to meet treatment requirements. TMPs were most difficult to remove, and more research is needed to find a way to optimize the their removal from the contaminated water. Little attention has previously been paid to DMPs and TMPs, but their persistent character suggests that they should be included in studies involving creosote-contaminated water in the future.

Biological processes were important in order to achieve low outlet concentrations and will likely extend the lifetime of the barrier. However, sorption to organic material might make hydrophobic compounds unavailable for microorganisms, hence, sorption sites are filling up. A two-zone barrier is suggested in order to enhance biological removal; the first zone contains minimum of organic material so that the compounds are bioavailable for degradation, and the second zone contain sufficient organic material (e.g. peat) to sorb or further degrade the creosote compounds.

The microbial population seemed to have adapted rapidly to contaminant load, and there was no obvious lag time before degradation started in the columns. Hence, if degrading microorganisms are present in the contaminated groundwater, the biologically active barrier might be efficient soon after installation. As long as the barrier is fed contaminated water containing oxygen, the number of degrading organisms is relatively stable, indicating stable treatment efficiencies. Supply of oxygen and nutrients might be required in a full-scale barrier.

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References

[1] A.R. Gavaskar, N.J. Gupta, B.M. Sass, R.J. Janosy, D. O'Sullivan, Permeable Barriers for Groundwater Remediation—Design, Construction, and Monitoring, Battelle Press, Columbus, OH, 1998.
[2] D. Forget, L. Deschênes, D. Karamanev, R. Samson, in: A. Leeson, B.C. Alleman (Eds.), Proceedings of the 4th International Conference on In Situ and On-site Bioremediation, Vol. 4, New Orleans, LA, 1997, Battelle Press, Columbus, OH, 1997, pp. 221–226.
[3] L. Yerushalmi, M.F. Manuel, S.R. Guiot, Biodegradation 10 (1999) 341.
[4] C.M. Kao, L. Yang, Water Sci. Technol. 42 (2000) 429.
[5] C.M. Kao, R.C. Borden, J. Environ. Eng. 123 (1997) 18.
[6] H.H.M. Rijnaarts, A. Brunia, M. van Aalst, in: A. Leeson, B.C. Alleman (Eds.), Proceedings of the 4th International Conference on In Situ and On-site Bioremediation, Vol. 4, New Orleans, LA, 1997, Battelle Press, Columbus, OH, 1997, pp. 203–208.
[7] United States Environmental Protection Agency, In Situ Remediation Technology Status Report: Treatment Walls, EPA/542/K-94/004, 1995.
[8] R. Borden, R. Goin, C.M. Kao, Groundwater Monitoring and Remediation Winter, 1997, p. 70.
[9] M. Warith, L. Fernandes, N. Gaudet, Waste Manage. 19 (1999) 9.
[10] K.J. Cantrell, D.I. Kaplan, T.W. Wietsma, J. Hazard. Mater. 42 (1995) 201.
[11] C.M. Su, R.W. Puls, Environ. Sci. Technol. 35 (2001) 1487.
[12] S.J. Morrison, D.R. Metzler, C.E. Carpenter, Environ. Sci. Technol. 35 (2001) 385.
[13] D.R. Burris, K. Hatfield, N.L. Wolfe, J. Environ. Eng. 122 (1996) 685.
[14] S. Benner, D. Blowes, C. Ptacek, Groundwater Monitoring and Remediation Fallm, 1997, p. 99.
[15] M.J. Brough, A. Al-Tabbaa, R.J. Martin, in: A. Leeson, B.C. Alleman (Eds.), Proceedings of the 4th International Conference on In Situ and On-site Bioremediation, Vol. 4, New Orleans, LA, 1997, Battelle Press, Columbus, OH, 1997, pp. 233–237.
[16] N.W. Bowles, L.R. Bentley, B. Hoyne, D.A. Thomas, Groundwater 38 (2000) 172.
[17] G.R. Brubaker, B. Symons, M. Westray, G. Kuntz, in: Subsurface Barrier Technologies, Tucson, AZ, International Business Communications, Southborough, MA, 1998.
[18] D.J. Katic, J.F. Devlin, J.F. Barker, M.L. McMaster, M.J. Brown, in: A. Leeson, B.C. Alleman (Eds.), Proceedings of the 4th International Conference on In Situ and On-site Bioremediation, Vol. 4, New Orleans, LA, 1997, Battelle Press, Columbus, OH, 1997, pp. 255–260.
[19] J.G. Johnson, J.E. Odencrantz, in: A. Leeson, B.C. Alleman (Eds.), Proceedings of the 4th International Conference on In Situ and On-site Bioremediation, Vol. 4, New Orleans, LA, 1997, Battelle Press, Columbus, OH, 1997, pp. 215–220.
[20] D.W. Hyndman, M.J. Dybas, L. Forney, R. Heine, T. Mayotte, M.S. Phanikumar, G. Tatara, J. Tiedje, T. Voice, R. Wallace, D. Wiggert, X. Zhao, C.S. Criddle, Groundwater 38 (2000) 462.
[21] C.M. Kao, S.C. Chen, J.K. Liu, Chemosphere 43 (2001) 1071.
[22] J.G. Mueller, P.J. Chapman, P.H. Pritchard, Environ. Sci. Technol. 23 (1989) 1197.
[23] E. Arvin, E.M. Godsy, D. Grbic-Galic, B. Jensen, in: Y.C. Wu (Ed.), International Conference on Physiochemical and Biological Detoxification of Hazardous Waste, Atlantic City, NJ, Technomic, Lancaster, PA, 1988, pp. 828–847.
[24] M.L. Richardson, S. Gangolli, The Dictionary of Substances and Their Effects, Royal Society of Chemistry, England, 1992.
[25] M.M. Broholm, Retardation and Degradation of Coal-tar Compounds in Fractured Geologic Media, Ph.D. Dissertation, Department of Environmental Science and Engineering, Technical University of Denmark, Denmark, 1998.
[26] S. Dyreborg, Microbial Degradation of Water-soluble Creosote Compounds, Ph.D. Dissertation, Department of Environmental Science and Engineering, Technical University of Denmark, Denmark, 1996.
[27] C. Cerniglia, Biodegradation 3 (1992) 351.

[28] D. Millette, J.F. Barker, Y. Comeau, B.J. Butler, E.O. Frind, B. Clément, R. Samson, Environ. Sci. Technol. 29 (1995) 1944.
[29] C.T. Chiou, in: B.L. Sawhney, K. Brown (Eds.), Reactions and Movement of Organic Chemicals in Soils, SSSA Special Publication No. 22, Soil Science Society of America Inc., American Society of Agronomy Inc., Madison, WI, 1989.
[30] M.M. Broholm, K. Broholm, E. Arvin, J. Contam. Hydrol. 39 (1999) 201.
[31] J.J. Pignatello, in: B.L. Sawhney, K. Brown (Eds.), Reactions and Movement of Organic Chemicals in Soils, SSSA Special Publication No. 22, Soil Science Society of America Inc., American Society of Agronomy Inc., Madison, WI, 1989.
[32] S.J. Allen, in: G. McKay (Ed.), Use of Adsorbents for the Removal of Pollutants From Wastewaters, CRC Press, New York, 1996, p. 59.
[33] G. Rasmussen, H. Iversen, Andersen S., in: Proceedings of the 30th Annual Meeting for the International Research Group on Wood Preservation. Section 5. Environmental Aspects, IRG/WP 99-50141, Rosenheim, Germany, IRG Secretariat, Stockholm, Sweden, 1999, p. 14.
[34] S.E. Bailey, T.J. Olin, R.M. Bricka, D.D. Adrian, Water Res. 33 (1999) 2469.
[35] D. Couillard, Water Res. 28 (1994) 1261.
[36] T. Viraraghavan, A. Ayyaswami, Can. J. Civil Eng. 14 (1987) 230.
[37] A.D. Cohen, M.S. Rollins, W.M. Zunic, J.R. Durig, Water Res. 25 (1991) 1047.
[38] T. Viraraghavan, S. Tanjore, Hazard. Waste Hazard. Mater. 11 (1994) 423.
[39] T. Viraraghavan, F.D. Alfaro, J. Hazard. Mater. 57 (1998) 59.
[40] M. Sparrevik, G.D. Breedveld, Land Contam. Reclamation 8 (2000) 1.
[41] O. Grahl-Nielsen, B. Landgren-Skjellerudsveen, in: Proceedings of the Report of the Workshop of the Working Party 8, Commission of the European Communities, Dübendorf, Switzerland, 1982.
[42] R.T. Noble, J.A. Fuhrman, Aquat. Microbial. Ecol. 14 (1998) 113.
[43] R.C. Weast, M.J. Astle, CRC Handbook of Chemistry and Physics, 60th Edition, CRC Press, Boca Raton, FL, 1980.
[44] P. Kjeldsen, T.H. Christensen, Kemiske stoffers opførsel i jord og grundvand (in Danish), Miljø-og Energiministeriet Miljøstyrelsen, Copenhagen, Denmark, 1996.
[45] G. Sundström, A. Larsson, M. Tarkpea, in: O. Hutzinger (Ed.), Anthropogenic Compounds, Springer, Berlin, Germany, 1986, p. 159.
[46] K.L.E. Kaiser, V.S. Palabrica, Water Pollut. Res. J. Can. 26 (1991) 361.
[47] B. Sharak Genthner, G. Townsend, S. Lantz, J. Mueller, Arch. Environ. Contam. Toxicol. 32 (1997) 99.
[48] J. Flyvbjerg, Anaerobic Degradation of Creosote-related Aromatic Compounds Under Nitrate-reducing Conditions, Ph.D. Dissertation, Department of Environmental Science and Engineering, Technical University of Denmark, 1992.
[49] D.L. McNally, J.R. Mihelcic, D.R. Lueking, Environ. Sci. Technol. 32 (1998) 2633.
[50] D. Licht, Anaerobic Degradation of Heterocyclic Compounds From Creosote, Ph.D. Dissertation, Department of Environmental Science and Engineering, Technical University of Denmark, 1995.
[51] J. Mueller, S.E. Lantz, B.O. Blattmann, P.J. Chapman, Environ. Sci. Technol. 25 (1991) 1055.
[52] E. Arvin, B.K. Jensen, A.T. Gundersen, Water Sci. Technol. 23 (1991) 1375.
[53] M.M. Broholm, E. Arvin, J. Contam. Hydrol. 44 (2000) 239.
[54] J.P. Arcangeli, E. Arvin, Water Sci. Technol. 31 (1995) 117.
[55] A.O. Thomas, J.N. Lester, Hazard. Waste Hazard. Mater. 10 (1993) 413.
[56] S.A. Boyd, Soil Sci. 134 (1982) 337.
[57] O. Ceyhan, H. Guler, R. Guler, Adsorp. Sci. Technol. 17 (1999) 469.
[58] B.S. Xing, W.B. McGill, M.J. Dudas, Y. Maham, L. Hepler, Environ. Sci. Technol. 28 (1994) 466.
[59] S.W. Karickhoff, D.S. Brown, T.A. Scott, Water Res. 13 (1979) 241.
[60] T. Larsen, T.H. Christensen, M. Brusseau, Chemosphere 24 (1992) 141.
[61] K.M. Scow, C.R. Johnson, Adv. Agron. 58 (1997) 1.
[62] K.A. Allan, B. Herbert, P.J. Morris, T.J. McDonald, in: A. Leeson, B.C. Alleman (Eds.), Proceedings of the 4th International Conference on In Situ and On-site Bioremediation, Vol. 5, New Orleans, LA, 1997, Battelle Press, Columbus, OH, 1997, pp. 629–635.
[63] G.W. Bailey, J.L. White, T. Rothberg, Soil Sci. Soc. Am. Proc. 32 (1968) 222.
[64] J.M. Zachara, C.C. Ainsworth, C.E. Cowan, B.L. Thomas, Environ. Sci. Technol. 21 (1987) 397.

[65] A.B. Thomsen, K. Henriksen, C. Gron, P. Moldrup, Environ. Sci. Technol. 33 (1999) 2891.
[66] E. Arvin, B. Jensen, J. Aamand, C. Jørgensen, Water Sci. Tech. 20 (1988) 109.
[67] S. Dyreborg, E. Arvin, K. Broholm, Biodegradation 7 (1996) 191.
[68] S. Dyreborg, E. Arvin, K. Broholm, Biodegradation 7 (1996) 97.
[69] S. Meyer, H. Steinhart, Chemosphere 40 (2000) 359.
[70] S. Dyreborg, E. Arvin, K. Broholm, J. Christensen, Environ. Toxicol. Chem. 15 (1996) 2290.
[71] B.J. Butler, S.E. Barbaro, F.H. Crocker, C.I. Mayfield, Geomicrobiol. J. 14 (1997) 253.
[72] G.D. Breedveld, M. Sparrevik, Biodegradation 11 (2001) 391.
[73] S.E. Barbaro, H.J. Albrechtsen, B.K. Jensen, C.I. Mayfield, J.F. Barker, Geomicrobiol. J. 12 (1994) 203.