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Effects of high sediment on biodegradation

Scientists from Beijing Normal University in Beijing, China examined the effects of high sediment levels in the Yellow River, the world's most turbid large river, on the biodegradation of three polycyclic aromatic hydrocarbons (PAH). The PAHs under study were chrysene, benzo(a)pyrene and benzo(g,h,i)perylene with phenanthrene "as a co-metabolism substrate in natural waters from the Yellow River" (Xia and others, 2006). The researchers sought to learn the effects of sediment on PAH fate and biodegradation with particular respect to indigenous bacteria in natural waters.

Xia and others (2006) determined the biodegradation kinetics of the Yellow River water with various levels of sediment contents. They also investigated the mechanisms related to biodegradation by "comparing the growth of bacteria in the water systems with various contents of sediment, and by examining bacteria and PAH distribution in water and solid phases." PAHs likely originated from coal burning in the river basin, as well as from the combustion of petroleum.

Materials and methods

Study site

The Yellow River had an average suspended sediment content of 28 g/L, with a range of 0 to 15 g/L in samples taken at the Huayuankou Hydrological Station, located in the middle reaches of the river. Previous research by Xia et al (2004) indicated that benzo(a)pyrene concentrations in the "middle and lower reaches of the river exceeded its environmental quality standard for surface water. The concentration of PAHs in some tributaries of the river reached 1 µg/L."

Researchers took water and sediment samples at a depth of 0.2 m in the central stream point on December 10, 2003 and February 20, 2004. Samples were then placed in a cooler and taken to a laboratory for analysis (Xia and others, 2006).

Medium and cultivation

Researchers used water and sediment samples as media for biodegradation experiments. They added a PAH methanol stock solution containing phenanthrene, chrysene, benzo(a)pyrene and benzo(g,h,i)perylene to Erlenmeyer flasks. Water samples collected in December of 2003 contained 10, 3.80, 1.12 and 2.35 µg/L, respectively, of phenanthrene, chrysene, benzo(a)pyrene and benzo(g,h,i)perylene. Samples collected in February of 2004 contained 25, 36, 5.34 and 7.28 µg/L of phenanthrene, chrysene, benzo(a)pyrene and benzo(g,h,i)perylene, respectively. Xia and others (2006) then evaporated the methanol in a fume hood and added 100 mL of the water sample with a known amount of sediment to each flask. The flasks were covered and placed in an orbital shaking incubator to promote PAH-degrading microorganism growth as well as the interactions between water and sediment. Researchers replaced water lost during cultivation every three days to keep a constant 100 mL volume. They duplicated each experimental set with controls and examined PAH and PAH-degrading bacteria in water and solid phases at regular time intervals... Since the suspended sediment content ranged from 0 to 15 g/L in the Huayuankou Hydrological Station, the studied levels of sediment content were 0, 4 and 10 g/L in this research" (Xia and others, 2006).

Sorption-desorption

Researchers added mixed PAH standard stock solutions to beakers for the sorption experiment. They put river water and sediment in each, along with 0.1% sodium

azide. The researchers examined PAH concentrations of each beaker before placing each in an orbital shaking incubator, where they were left for 48 hours. Xia and others (2006) centrifuged each beaker and then performed the following:

- decanted supernatants for PAH analyses
- calculated PAH amount in solid
- added 100 mL river water with 0.1% sodium azide to each sorption system with only the solid phase
- shook flasks for another 48 hours and determined the equilibrium concentration of PAHs in the water phase

Analysis

For chemical analysis, the scientists extracted water and solid-phase PAHs with cyclohexane, dehydrated, concentrated and dissolved in methanol. They analyzed the PAHs with high performance liquid chromatography (HPLC) with a fluorescence detector. They also analyzed the microbial properties of PAHs by determining the densities of PAH-degrading bacteria in water with a most probable number (MPN) technique. They added the PAH mixture solution with *n*-hexane to each well of the microtiter plate. The mixture consisted of the following:

- 2 g/L phenanthrene
- 1.5 g/L chrysene
- 1 g/L benzo(a)pyrene
- 1 g/L benzo(g,h,i)perylene

The scientists then evaporated the *n*-hexane and added 200 µL Bushnell-Haas medium supplemented with 0.85% NaCl to each well as the growth medium. The samples were diluted with 0.85% sterilized NaCl solution. Tenfold serial dilutions were completed, followed by inoculations, leaving one row of wells uninoculated as controls and one row inoculated with the appropriate sample dilution but without PAHs.

Scientists wrapped the plates and incubated them for three weeks at room temperature before adding indonitro-tetrazolium violet (INT) to each well and incubating them again for 18-24 hours. They judged bacteria growth by turbidity and "the appearance of a purple color. The color change indicated the reduction of INT by bacteria respiring in this well, and that well was judged as a positive well. Positive wells were scored and density of bacteria was calculated by using MPN tables for the five-tube format" (Xia and others, 2006).

Results

Water and sediment characteristics

The Yellow River water sample had the following characteristics:

- 365.8 mg/L total ion content
- slightly alkaline
- PAH background concentrations < 1.0 µg/L
- 78.6% of sediment grains were composed of particles 0.02-0.25 mm in size

Phenanthrene, used as a co-metabolism agent for the experiment, was determined to differ insignificantly in amount in systems with different sediment contents. The sediment was not small enough to be suspended in water unless shaken at 125 rpm or higher. Therefore, most sediment existed as deposited sediment while a small part existed as suspended particles during cultivation. Its organic matter content was reasonably low at 0.45%.

Biodegradation

Xia and others (2006) observed the following for samples with chrysene taken in December of 2003:

- insignificant loss of chrysene from abiotic processes during control experiments
- rapid reduction of chrysene, when sediment contents

were 0, 2 and 4 g/L only after 3 days of cultivation during control experiments

- rapid reduction of chrysene, when the sediment content was 10 g/L, from the beginning of cultivation
- higher biodegradation rates of chrysene in water systems with sediment than those without sediment during cultivation
- increased rates of chrysene biodegradation with the sediment content in the initial phase of cultivation
- insignificant difference among biodegradation rates of chrysene in water with different sediment contents after 13 days of cultivation
- slow decrease in chrysene after cultivation for 20 days in all water systems

Samples collected in February of 2004 contained 36 µg/L chrysene initially and showed an increasing trend in the biodegradation rate, from a water system with 0 g/L sediment to 4 g/L sediment to 10 g/L sediment.

The researchers observed increased biodegradation rates for benzo(a)pyrene and benzo(g,h,i)perylene with the sediment content in the water system.

Bacteria

Xia and others (2006) observed that in a water system with a 4 g/L sediment content, the density of PAH-degrading bacteria in the sediment phase, 3.5×10^5 cell/mL, was 3.5 times higher than that in the water phase during the cultivation. They reported, "The peak value of bacteria density in the sediment phase was about 1.1×10^6 cell/mL, which was approximately two times of that in the water phase."

The researchers also found that PAH-degrading bacteria populations increased with sediment content in the water system. The bacteria increased rapidly at the beginning of cultivation for all water systems,

reached peak value after a 2-week cultivation, and then gradually decreased. "The peak value of bacteria density in the water system with the sediment content of 10 g/L was about 16 times of that without sediment and three times of that with the sediment content of 4 g/L" (Xia and others, 2006).

Biodegradation kinetics

Scientists fitted the biodegradation kinetics of chrysene, benzo(a)pyrene and benzo(g,h,i)perylene to a model for the kinetics of biodegradation of organic compounds not supporting growth. "In addition, since the PAH-degrading bacteria grew with an exponential function and the initial concentrations of PAHs were relatively low in the water system, an exponential model was applied to analyze the kinetics of PAH biodegradation." They determined after calculation that for chrysene and benzo(a)pyrene, the biodegradation rate constant increased with the sediment content, no matter the initial concentrations. Benzo(g,h,i)perylene could not be analyzed with the kinetics model because it was too slow during the cultivation period.

Sorption-desorption and biodegradation

The scientists discovered that PAH per unit weight of sediment sorption capacity decreased with increased sediment content. They also found that "for a given quantity of PAHs sorbed on sediment, the corresponding equilibrium concentrations of PAHs in the water phase reached in the sorption process were much higher than that in the desorption process." Thus, they determined that approximately "75% of the total PAH-degrading bacteria and 90% of the total PAHs in the water system attached on the sediment phase, and the biodegradation rates of PAHs increased with the sediment content. This suggested that biodegradation of PAHs mainly occurred on the sediment phase. Therefore, the sorption-desorption process of PAHs on the solid phase would exert influences on the

biodegradation." They also determined that "the population of PAH-degrading bacteria in the water phase was far lower than that in the sediment phase and did not maintain at a stable level. Therefore, the biodegradation of PAHs in the water system was not limited to bacteria in the water phase" (Xia and others, 2006).

The researchers found that PAH reduction in the sediment phase was much higher than in the water phase. They observed that concentrations of benzo(a)pyrene during the water phase "decreased quickly in the first week, which was caused by the rapid increase of PAH-degrading bacteria in the water phase at that time. After cultivation for approximately 10 days, the concentration of benzo(a)pyrene in the water phase kept at a level of 0.1 $\mu\text{g/L}$, which was less than the equilibrium concentration reached in the desorption process." The researchers reasoned that this may have been due to PAH-degrading bacteria's forming of wrapping biofilm on the surface of solids after cultivation, which would then consume PAHs desorbed from the solid phase (Xia and others, 2006).

Conclusions

Xia and others (2006) concluded the following:

- PAH biodegradation rates increased with sediment content in a water system and the biodegradation rate constants of chrysene and benzo(a)pyrene increased with the sediment content.
- "the growth of PAH-degrading bacteria fit the exponential equation in all water systems during the initial stage of cultivation and the bacteria growth rate increased with the sediment content. The population of PAH-degrading bacteria on the sediment phase was far greater than that in the liquid phase."

- PAH sorption on sediment could be described as a dual adsorption-partition model for which "equilibrium concentrations in the water phase reached in the sorption process were much higher than that in the desorption process."
- sediment-influenced mechanisms included PAH tendency to be sorbed during the sediment phase; "the desorption of PAHs from the solid phase led to a higher concentration of PAHs near the interface between water and sediment;" the attachment of bacteria to the interface between water and sediment resulted in increased contact chances between bacteria and PAHs, which in turn led to increased PAH biodegradation with sediment content in a water system.

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Direct-push tools for predicting MTBE plume diving

Researchers evaluated two site characterization techniques, electrical conductivity logging and pneumatic slug testing, used to predict the vertical extent of MTBE in an aquifer. These techniques were evaluated as performed in temporary push wells.

Site description

The site under investigation was an aquifer that supplies water to the village of East Alton, Illinois. The aquifer had been contaminated with MTBE. The most plausible sources were two nearby service stations northeast of the municipal supply wells. The Illinois EPA investigated the site in 1999. There was less than 500 m between the supply wells and the service stations. The first 250 m between the plausible sources and the wellfield were paved or covered with buildings. Shallow wells placed in the first 100 m of the flowpath detected the plume, while deeper wells, 6.5 to 12.5 m below the water table, were required to detect MTBE in the second 100 m. "Because there was minimal opportunity for recharge through the paving, burial of the MTBE plume by recharge could not explain the absence of MTBE in the shallow wells" (Wilson and others, 2005).

Wilson and others (2005) reported that the Illinois EPA employed electrical conductivity logging with push technology to characterize the hydrostratigraphy of the aquifer. The site southwest of pavement was characterized by a layer of silt and clay extending up to 7 m into the aquifer. Below the silt and clay was a layer of sand and gravel. The Illinois EPA workers were able to locate the plume by sampling water from the layer of sand and gravel.

U.S. EPA workers repeated and extended electrical conductivity logging. "The vertical distribution of hydraulic conductivity in the aquifer was defined with a downhole flowmeter survey in a well that was screened across most of the aquifer." The workers evaluated the correspondence between hydrostratigraphy and hydraulic conductivity by comparing an electrical conductivity log to the vertical distribution of hydraulic conductivity.

U.S. EPA workers performed the following:

- compared hydraulic conductivity obtained from pneumatic tests to downhole flowmeter test
- defined the vertical distribution of MTBE
- evaluated inferences about the texture of aquifer material

Wilson and others (2005) noted that plume diving may be controlled by recharge to the aquifer or by aquifer hydrostratigraphy. The U.S. EPA workers considered the possibility that the MTBE plume could undergo natural biodegradation along the flowpath as well as the possibility that MTBE would "persist along the flowpath from the potential source areas to the municipal wells."

Site history

The Illinois EPA reported that in October of 1999, MTBE was detected in water supply wells 6, 8, and 9 within the East Alton wellfield, on the east side of the Wood River. In January of 2000, monitoring wells near the release were sampled. The maximum concentration of MTBE was 2200 µg/L. In June 2000 the site of the leaking UST was excavated of approximately 13,000 tons of contaminated materials and replaced with clean fill. When sampled, the maximum concentrations of MTBE in East Alton water supply wells 6, 8, and 9 were 32, 61, and 560 µg/L, respectively.

Ground water flow

The ground water flowed, in general, from the potential source, under a shopping mall, and then under a grassy undeveloped area to the municipal water supply wellfield. U.S. EPA workers, after three round of tests, found the following:

- ground water flow varied by 23°
- hydraulic gradient varied by 45%
- the water table could be approximated by a plane

Wilson and others (2005) suspected that the MTBE "that first reached the water supply wells would have moved through the most conductive material in the aquifer." If this were true, the MTBE plume near the possible source areas would be moving toward the municipal wellfield between 86 to 133 m/year (Wilson and others, 2005).

Illinois EPA evidence for plume diving

The Illinois EPA workers mapped the MTBE plume distribution by sampling permanent wells northwest of the shopping center and by using push technology to sample ground water southwest of the shopping center. Permanent wells were installed with screens at depths ranging from 9.1 to 10.7 m below land surface. From temporary push wells southwest of the shopping mall the workers took water samples at depths of 9, 15, and 21 m below ground surface or 0.5, 6.5, and 12.5 m below the water table.

MTBE was detected in shallow ground water northeast of the shopping mall but not southwest. MTBE was detected in the southwest area at depths of 6.5 and 12.5 m below the water table. Wells near the center of the plume contained concentrations of MTBE > 1000 µg/L. "The area above the plume of MTBE was paved or roofed. There is little possibility that recharge could explain the diving plume." Illinois EPA workers

used an electrical conductivity probe mounted on direct-push tools "to better understand the influence of hydrostratigraphy on the behavior of the plume of MTBE." They found that "the plume of MTBE had 'dived' below the elevation of the shallow ground water samples as it followed the flow of ground water in the aquifer" (Wilson and others, 2005).

Material and methods

Wilson and others (2005) used the work of the Illinois EPA as a basis for further characterization of the flowpath between the possible sources of MTBE and the impacted water production wells. They performed the following for data collection:

- acquired electrical conductivity logs and water samples from permanent and temporary wells
- preserved volatile organic compound (VOC) samples with 1% trisodium phosphate
- determined MTBE, *tert*-butyl ether (TBA), and BTEX concentrations by gas chromatography and mass spectrometry
- prepared methane, sulfate, chloride, nitrate and nitrite samples

The scientists conducted pneumatic slug tests on discrete depth intervals by advancing temporary push wells into the aquifer. They performed the tests on two permanent monitoring wells. Screens of the wells were 1.1 m long and 3.8 cm in diameter. A sheath protected each screen while the push well was advanced into the aquifer. The scientists pulled back the sheath to present the screen to the aquifer material and returned the sheath when the tools were retrieved. Water was depressed with compressed air during each test, released when the well came to equilibrium with the air pressure. They used a pressure transducer and data logger "to monitor the water level over time

as it returned to its original level." Multiple tests were conducted using different water displacements. The data was then analyzed (Wilson and others, 2005).

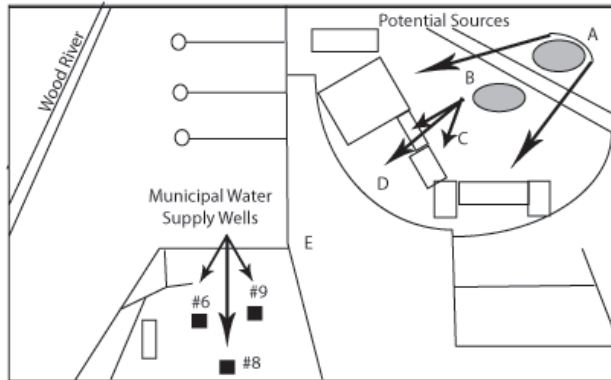


Figure 1 (not to scale): Sampling locations (from Wilson and others, 2005)

Researchers then defined the vertical distribution of hydraulic conductivity of the location D, shown in Figure 1. At location D, the plume had moved 10.7 m below the water table, 19.8 m below land surface. They installed a test well, 5.1 cm in diameter and screened from 11.6 to 25.3 m below land surface, and measured the vertical component of ground water flow in the well "at multiple intervals under undisturbed (ambient) conditions and during water injection at a constant rate of 10 L/min. The rate of water flow from the well into an individual interval during steady-state injection or extraction is proportional to the hydraulic conductivity of the materials adjacent to the screen. Therefore, knowledge of the distribution of flow at each measurement interval along the screen allows interpretation of the hydraulic conductivity distribution relative to the average hydraulic conductivity of materials screened by the well" (Wilson and others, 2005).

Researchers analyzed the vertical flow rates under ambient conditions and constant rate conditions. They

subtracted the ambient flow rate from the measured flow rate to determine the differences in horizontal flow into the formation due to differences in hydraulic conductivity of aquifer materials and hydraulic gradients. They also used pneumatic slug tests conducted in temporary push wells to determine the average hydraulic conductivity (Wilson and others, 2005).

Changes in hydrostratigraphy

Wilson and others (2005) characterized the hydrostratigraphy along an inferred flowpath extending from the potential sources of MTBE contamination to the contaminated water supply wells. They found the following:

- MTBE plume in sandy sediment near the water table at locations A, B, and C
- a layer of clay and silt at locations D and E, extending from the water table 9 m and 5.2 m, respectively, into the aquifer
- MTBE plume that dived below the layer of clay and silt at location D and E

Electrical and hydraulic conductivity

The researchers determined that location D was the first location along the flowpath where the MTBE plume had dived below the water table. The pneumatic slug test showed an average hydraulic conductivity of 14.3 m/d. They found the following hydraulic conductivity values:

- at an elevation of 111.3 m, 51.2 m/d
- at an elevation of 114.3 m, 0.27 m/d
- at any elevation greater than 114.3 m, 0.4 m/d or less

Wilson and others (2005) determined that "99% of the transmissivity in the interval between elevations of 120.4 and 106.7 m amsl was associated with the sandy material that extended between 114.0 and 106.7 m amsl."

Plume diving predicted from hydrostratigraphy

The scientists determined that, along the flowpath, concentrations of MTBE were highest in association with sandy material with low electrical conductivity and were found near the water table of potential sources, location B. Where the plume first dived below the water table, the highest concentrations were found at the contact between the shallow clay unit and the underlying sand unit, location D. At location E, the highest concentration of MTBE was found more to the center of the sandy unit. They determined that the MTBE plume followed the hydrostratigraphic units with highest hydraulic conductivity (Wilson and others, 2005).

MTBE biodegradation

Wilson and others (2005) reported that MTBE persisted along the flowpath while BTEX was depleted. They observed that the ground water was "essentially devoid of oxygen and nitrate, contained little methane, and the concentration of TBA was below the detection limit, which was much lower than the concentrations of MTBE. Based on the geochemical environment, MTBE should degrade slowly or not at all in the ground water at East Alton. Because it persists, there is an opportunity for the MTBE to move with the flow of ground water to the water supply wellfield."

Conclusions

The scientists concluded that the MTBE plume movement below the water table at East Alton appeared as though it had dived into the aquifer. Biodegradation of MTBE was prevented by the geochemistry of the ground water. Also, "the hydrostratigraphy of the aquifer controlled the vertical distribution of MTBE contamination. The plume of MTBE simply followed the natural flow of ground water. This study validated the conceptual model of the diving MTBE plume that was developed by the Illinois EPA" (Wilson and others, 2005).

Wilson and others (2005) reported, "The downhole flowmeter test was conducted as a research activity to provide a benchmark for the electrical conductivity log and the pneumatic slug tests. Strictly speaking, flowmeter tests require a fully screened well across the aquifer of interest. However, such wells are expensive and are rarely available at UST release sites. The pneumatic slug test offers a realistic alternative for mapping the vertical distribution of hydraulic conductivity, and identifying the optimum depth intervals for taking push samples or locating screens for permanent monitoring wells... Pneumatic slug testing is well developed and can be considered a routine tool for site characterization" because it is cost effective. They noted, "During this investigation, a two-person crew set up the equipment for electrical conductivity logging, logged 80 feet of subsurface material, and then recovered and cleaned the tools in an average of 2 hours."

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Thermally enhanced bioremediation

Professor Banat and co-workers at the University of Ulster, UK (Perfumo and others, 2006; Perfumo, Banat and Marchant, 2006) described two main approaches

to bioremediation. These include

- environmental biostimulation, where oxygen and/or mineral nutrients are added
- bioaugmentation, where a degrader microorganism is applied directly

They noted that temperature is among several factors that affect the rate of microbial degradation of hydrocarbons in soil because it contributes to the "bioavailability of low-solubility hydrocarbons and hence the nature and the extent of microbial metabolism. The rate at which microbial cells can convert contaminants during bioremediation depends on the rate of contaminant uptake and metabolism and the rate of transfer to cell (mass transfer). Increasing temperature leads to a decreased viscosity, higher solubility and faster diffusion of hydrophobic contaminants to the cell, thus enhancing the biodegradation rates." Thus, they investigated the effect of high temperature in enhancing hydrocarbon degradation. They focused upon bioremediation as a remediation strategy (Perfumo and others, 2006).

Materials and methods

Researchers took soil samples from an uncontaminated area in Northern Ireland. The samples were sieved and divided into two parts. One part was sterilized at 121°C for 30 minutes and both were then dried overnight at 60°C.

Perfumo and others (2006) conducted experiments examining the five following bioremediation techniques:

- natural attenuation, in which "microcosms containing non-sterile soil (NSS) + hydrocarbon (HY) were left untreated and monitored for the degradation potential by indigenous microorganisms"
- biostimulation, in which "a nitrogen phosphorus potassium solution was supplied into microcosms at 0.5% (v/v) concentration (NSS + HY + NPK)"

- biosolubilization, in which "a 0.5% (v/w) rhamnolipids solution (15 g L⁻¹) was added to microcosms (NSS + HY + RL) in order to increase the bioavailability of hydrocarbon
- bioaugmentation, in which "microcosms were inoculated with 500 µL of a selected hydrocarbon-degrading bacterial suspension with an OD₍₆₀₀₎ 1.0 (NSS + HY + BI)"
- biostimulation + biosolubilization + bioaugmentation, in which "microcosms were prepared with all the above amendments together (NSS + HY + NPK + RL + BI)"

They conducted microcosm experiments in sterile screw cap glass universal bottles containing 5 g of soil artificially contaminated with 2% (v/w) *n*-hexadecane. Sterile soil with no amendments (SS + HY) was used as abiotic controls. The scientists adjusted water content to 30% (w/w) by adding sterile water. They investigated the effect of high temperatures on degradation by incubating the microcosms at both 60°C and room temperature. The microcosms were monitored at 0, 5, 15, 30 and 40 days for the estimation of hydrocarbon content by gas chromatography and the bacterial population. The scientists conducted all treatments in duplicates.

Bacterial inoculum

Researchers used the bacterium *G. thermoleovorans* T80 for the bioaugmentation experiments. This was an ideal bacterium because it grew optimally at 60°C, was able to use a wide range of hydrocarbons as carbon sources and was indigenous to the soil under study. The researchers prepared bacterial inoculum "on nutrient broth at 60°C until stationary phase, centrifuged and resuspended in a saline solution (1 g L⁻¹ NaCl) to required optical density" (Perfumo and others, 2006).

Amendments

Scientists prepared a NPK solution by dissolving 10 g L⁻¹ of each of NH₄NO₃ and K₂HPO₄ in sterile water. Biosurfactant used was JBR 215 rhamnolipid solution containing 15% (w/v) rhamnolipids.

Hydrocarbon degradation

Perfumo and others (2006) used GID gas liquid chromatography to estimate the hexadecane degradation in the soil microcosms. They performed the following for the incubated microcosms:

- let stand at room temperature for at least 1 hour
- mixed carefully before opening and extracting
- used hexane to extract the hexadecane from soil (2:1 ratio)
- mixed soil and hexane for 30 minutes

They then recovered soil-free hexane extract and used it for analysis. The extraction of hexadecane from the soil was > 98% efficient.

The researchers reported that the conditions for gas chromatography included the following:

- injection temperature 250°C
- detector temperature 250°C
- column temperature at 50°C for 4 minutes, then increased at the rate of 20°C min⁻¹ to 330°C for 3 minutes

Analysis

The scientists conducted total bacterial counts of thermophilic and mesophilic bacteria in the soil microcosms at 0 and 40 days with serial dilution plating onto nutrient agar. Incubation was at either 60°C or room temperature. Five replicate plates were created.

Perfumo and others (2006) carried out a three-way analysis of variance to test the effect of bioremediation treatments "on hydrocarbon degradation rates at different times (0, 5, 15, 30 and 40 days) and at

different incubation temperatures (60°C and room temperature)." The analysis considered amendments, temperature and days of treatment. They replicated this twice. To test the effects of bioremediation on treatments, they tested the interaction terms for significance.

Results

Hydrocarbon degradation in soil

The researchers found the following in their examination of natural attenuation after 40 days of incubation for the polluted-soil-only microcosms:

- at 18°C, 30% of hexadecane degradation was measured
- at 60°C, 56% of hexadecane degradation was measured

They reported that the soil contained a microbial population "capable of supporting bioremediation" and their results "confirmed the ubiquity of hydrocarbon-degrading microorganisms. Interestingly, these data suggested the presence in such a cool soil of a community not only of mesophilic alkane degraders but also thermophilic degraders" (Perfumo and others, 2006).

For the soil treated with rhamnolipid biosurfactant, scientists found the following:

- significant hexadecane degradation and reduction, 71% at 60°C for 40 days
- significant hexadecane degradation and reduction, 42% at 18°C for 40 days

The researchers reported that the rhamnolipid molecules were able to increase bioavailability of hydrophobic contaminants by the "desorption of hydrocarbon from soil particles and solubilization into surfactant micelles. Since rhamnolipids are biodegradable and contain both sugar and lipid moieties, they may act

as an additional source of carbon contributing to the biodegradation enhancement" (Perfumo and others, 2006).

Perfumo and others (2006) also determined bioaugmentation to be a successful biodegradation technique, particularly when incubated at 60°C. They observed the following in this treatment after 40 days:

- at 60°C, 70% degradation of hexadecane in microcosms
- at room temperature, 38% degradation of hexadecane in microcosms

They explained that *G. thermoleovorans* T80 is inactive at temperatures below 40°C. Several other strains, "mainly belonging to *Thermus* and *Bacillus* genus, have been already isolated from hot environments and characterized for the ability to degrade hydrocarbons at 60-70°C especially in batch cultures, while to our knowledge this is the first report describing a thermally enhanced bioremediation by means of a thermophilic bacterium indigenous to a cool soil" (Perfumo and others, 2006).

The researchers found the greatest rates of biodegradation when all amendments were added together. They observed the following hexadecane degradation in this situation:

- 90% in the microcosms incubated at 60°C
- 48% in the microcosms incubated at 18°C

The results suggested that "increasing the temperature simultaneously to adding rhamnolipids could enhance dramatically the mass transfer of hexadecane, thus allowing its increased conversion and degradation by both indigenous microorganisms and inoculated T80" (Perfumo and others, 2006).

Microbes

The initial microbial population varied from:

- 5.6 to 9.5×10^5 cfu g⁻¹ in microcosms incubated at 18°C
- 1.2 to 6.5×10^4 cfu g⁻¹ in microcosms incubated at 60°C

The scientists observed a slight increase in bacterial population in some microcosms in both incubation groups. "In particular, rhamnolipid addition as well as fertilization with N, P and K had positive effects, suggesting these amendments were important in order to restore the bioavailability of essential micronutrients and thus the balance with the carbon content" (Perfumo and others, 2006).

Conclusions

Perfumo and others (2006) concluded that high temperature, 60°C, "significantly enhanced the degradation rates of hexadecane in soil microcosms compared to those at room temperature, leading to approximately twofold increased removal." They suggested that higher temperatures may further enhance contaminant removal from soil by mobilizing particles and increasing their solubility. "Moreover, the response of the indigenous microbial population at elevated temperature showed the activation of a community of thermophilic degraders, which suggested an intrinsic potential for natural attenuation in such cool soils through thermally enhanced bioremediation techniques."

They reported that, "the supplementation with limiting nutrients or augmentation with thermophilic geobacilli or addition of biosurfactants all contributed to additional degradation, resulting in a maximum of 90% removal of *n*-hexadecane within a 40-day period. Thermally enhanced bioremediation therefore may reduce in situ treatment time or be used as a complementary treatment to follow other physical techniques. Further investigation into the most cost-effective methods to provide the energy needed for soil heating and

augmentation procedures to stimulate the process is required" (Perfumo and others, 2006).

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UTTU thanks Dr. Ibrahim Banat, im.banat@ulster.ac.uk, for his help on this article.



Gasoline vapor intrusion in homes

Researchers investigated the potential for chemical vapor intrusion into homes from contaminated ground water. They examined the vapor intrusion pathway of petroleum-contaminated ground water. The site under study was an area near a gasoline station in Stafford, New Jersey, where a 4000-gallon gasoline tank was discovered to have been leaking. Ground water and soil contamination was revealed when the tank was removed. As shown below in Figure 2, downgradient from the source were several residential and commercial structures. The researchers reported that these structures had "a mixture of foundations including basements (extending to a depth of 1.7 m below the ground surface), slab-on-grade construction, and crawlspaces" (Sanders and Hers, 2006).

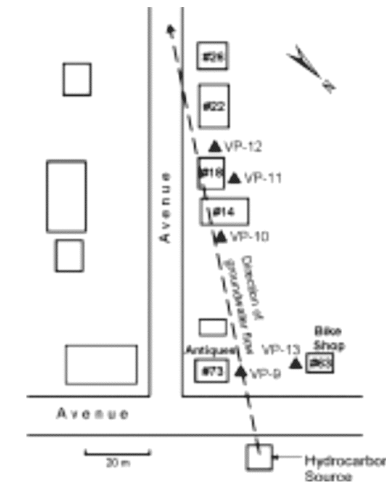


Figure 2: Diagram of area of study in Stafford Township, New Jersey (from Sanders and Hers, 2006).

The site, located above a large unconfined sand aquifer, consisted of unconsolidated sand through the whole vadose zone. The water table at the site was approximately 3.3 m below the surface, and ground water flow was 100 to 150 m/year to the south and southwest.

Methods

Researchers began sampling ground water, soil, soil vapor, and indoor and outdoor ambient air in October 2002. The contaminated area under investigation included three homes, buildings #14, #18, and #22, and two small commercial businesses, buildings #63 and #73. Sanders and Hers took ground water, soil and soil vapor samples 1 to 3 m laterally from each of the five structures on the same day while indoor air and subslab vapor samples were taken a few days later. Researchers collected meteorological data continuously from the Atlantic City National Weather Service located 39 km away, which was meteorologically similar to the site of study. The data included the following:

- atmospheric pressure
- temperature
- wind velocity
- wind direction
- precipitation

The researchers took soil cores from all five structures and screened for hydrocarbons at periodic depth intervals using a photoionization detector (PID) and a dry headspace vapor test. They took soil samples 3-m deep from the VP-9 and VP-13 soil cores for volatiles analysis, moisture retention tests and organic carbon analysis. Researchers took ground water samples by drawing the water through either stainless steel or polyvinyl chloride (PVC) Geoprobe® screens. They measured the pH and dissolved oxygen levels during purging. The water samples were then collected using a Waterra check valve and tubing.

Researchers used Geoprobe® Model AT86 implants for procuring soil vapor samples (15-cm-long by 1.3-cm-diameter cylindrical stainless steel screens). These were connected to Summa canisters with polyethylene tubing. The implants were installed 1, 2, and 3-m deep at locations VP-9 and VP-10, and 3-m deep at the other locations. The researchers surrounded the implants with sand and filled the remaining borehole with bentonite. The system was purged prior to sampling. "One-hour soil vapor samples were taken using 6-L Summa canisters. Samples were analyzed. Oxygen measurements were taken of the soil vapor" (Sanders and Hers, 2006).

Buildings were examined for possible storage sources of volatile organic compounds prior to indoor air sampling. No indoor gasoline storage was observed. The researchers took 24-h indoor, subsurface, and outdoor ambient air samples with 6-L Summa canisters. The samples were then analyzed. The structures had the

following characteristics and subsequent samples taken:

- #73, #14, and #18, concrete floor basements, basement and ground floor samples, subsurface vapor sample
- #73 and #18 also had partial crawlspaces open to the basement with exposed soil
- #22, earthen crawlspace, ground floor and crawlspace samples
- #63, slab-on-grade construction, ground floor sample, subsurface vapor sample

The researchers took the following differential pressure measurements using a differential pressure recorder:

- between basement and outdoor air for structures #73 and #14
- between subsurface and basement air for structure #14
- between crawlspace and indoor air for structure #22

Researchers also took ambient air samples adjacent to structures #73 and #22, 24-h, with 6-L Summa canisters and then analyzed these samples.

Results

Scientists reported environmental conditions to be near normal for the sampling period. Rainfall prior to the study had been preceded by two months of near-normal rainfall with no rainfall in the two days prior to sampling. They suggested that water table levels therefore may have been slightly below normal, though soil moisture levels "were expected to be nearly normal for the time of year sampled." The buildings sampled were occupied, so doors and windows were periodically opened (Sanders and Hers, 2006). Temperature ranges were

- -1°C to +4°C, night
- 7°C to 16°C, day

Temperatures were approximately 3°C cooler than normal. "Due to the cool weather, windows were largely, but not entirely, closed, and heating systems (natural gas-fueled forced air or oil-fueled hot water radiant) were under moderate use. The average daily wind speed during indoor air sampling at the Atlantic City weather station ranged from 2 to 6 m/s, and barometric pressure variations were moderate (1010 to 1030 mbar)" (Sanders and Hers, 2006).

Sanders and Hers (2006) reported that PID screening yielded the following soil core results:

- at depths 2.7- to 3.3 m, location VP-9, high levels of hydrocarbon vapors
- remaining VP-9 depths, most VP-13 and VP-10 depths "showed somewhat elevated vapor levels but these were not indicative of residual non-aqueous phase liquid (NAPL)"
- low vapor concentrations at VP-11 and VP-12
- above the water table, VP-9 confirmed contamination
- soil organic carbon content, 0.04% to 0.68%
- soil total porosity 0.35 to 0.4 (v/v), field capacity moisture 0.04 to 0.05 (v/v), residual water saturation 0.005 to 0.017 (v/v)

Ground water samples showed that BTEX concentrations existed in the milligram per liter range under structures #63, #73, and #14. Concentrations were significantly reduced below structures #18 and #22. MTBE concentrations were above 1 mg/L from the source to structure #18. The scientists found the ground water to be slightly acidic, pH 6.0 to 6.5, and to be a low oxygen environment with dissolved oxygen levels of 0.4 to 2.3 mg/L. The lowest oxygen concentrations were found near the contamination source.

For outdoor air samples, the scientists found

undetectable levels of benzene, ethylbenzene, MTBE, 2,2,4-trimethylpentane, and cyclohexane, with detection limits at or above commonly observed outdoor concentrations. They found $4 \mu\text{g}/\text{m}^3$ of toluene and xylene outside structure #22. The scientists noted that "elevated outdoor concentrations of these chemicals were not expected because Stafford Township is bounded on the east by the Atlantic Ocean and on the west by the Pine Barrens, a 30-mile-wide region of undeveloped land. Also, the gas station responsible for the ground water contamination had ceased operations prior to this study, so it did not represent a local aboveground source of gasoline vapors" (Sanders and Hers, 2006).

Gasoline tracers

Sanders and Hers (2006) reasoned that 2,2,4-trimethylpentane (isooctane) and MTBE are gasoline additives, uncommonly found in other sources, while BTEX chemicals are "commonly reported in indoor air and may have various sources. The appearance of gasoline-specific chemicals indoors may therefore serve as an indication that the indoor air has been impacted by gasoline." Therefore they additionally investigated 2,2,4-trimethylpentane.

Concentration patterns

For building #73, scientists found the following:

- MTBE, 590 mg/L in ground water
- VP-9, deep-soil vapor measurements: toluene, MTBE, 2,2,4-trimethylpentane, and cyclohexane were in excess of 1 million $\mu\text{g}/\text{m}^3$, with the highest observed concentration for MTBE ($5.9 \times 10^6 \mu\text{g}/\text{m}^3$); benzene was $660,000 \mu\text{g}/\text{m}^3$
- 1 m deep-soil vapor samples significantly lower than at 2 m deep
- only toluene of the BTEX chemicals was confirmed in the shallow soil vapor sample ($1000 \mu\text{g}/\text{m}^3$)

- oxygen levels were $< 1.0\%$ at the deep vapor sampling point, 0.9% to 1.6% at middepth, and 8% to 10% at shallow depth

The scientists determined that MTBE's attenuation was greater than for any of the four hydrocarbon chemicals. They also found that subslab concentrations of MTBE, 2,2,4-trimethylpentane, and cyclohexane "were lower than middepth-soil vapor concentrations adjacent to building #73, even though both these samples were taken at a similar depth (2 m)." They did not detect BTEX chemicals within the subslab vapor despite benzene and toluene detection in "substantial levels in the middepth-soil vapor sample" (Sanders and Hers, 2006).

Sanders and Hers (2006) discovered that the basement air and ground floor of structure #73 contained measurable levels of MTBE, 2,2,4-trimethylpentane, and cyclohexane. Evidence of a subsurface gasoline source included the following:

- a decreasing sequence of concentrations of contaminants in the ground water, deep-soil vapor, middepth-soil vapor, subslab vapor, and indoor air
- first-floor concentrations less than basement concentrations
- elevated levels of degradation-resistant MTBE, 2,2,4-trimethylpentane, and cyclohexane in the basement relative to the degradable BTEX compounds

In addition, scientists observed significant levels of toluene, ethylbenzene, and xylene in the ground floor.

For the other structures, scientists found the following:

- structures #63 and #14, underlying ground water contained milligrams per liter concentrations of gasoline constituents
- structure #63, deep-soil vapor contained high concentrations of contaminants, but low subslab and

indoor air concentrations

- structure #14, deep-soil vapor contained $> 1000 \mu\text{g}/\text{m}^3$ for toluene, MTBE, and 2,2,4-trimethylpentane, and in the middepth-soil vapor sample, several hundred micrograms per cubic meter were measured for MTBE, 2,2,4-trimethylpentane, cyclohexane, and all BTEX chemicals except benzene
- structure #14, low contaminant concentrations in shallow-soil vapor, subslab, basement, and indoor samples
- structure #14, subslab and basement differences in contaminant concentrations too low to suggest vapor intrusion
- structures #18 and #22 ground water: relatively low contaminant concentrations

Contaminant attenuation

For low ground water concentrations, scientists observed that deep soil vapor concentrations were frequently $< 1\%$ of predicted concentrations using the Henry's law constant. For higher ground water concentrations, at locations VP-9 and VP-13, deep soil vapor concentrations were between 10% and 50% of predicted concentrations. They noted, "higher percentages would be expected at these locations since residual NAPL was likely present above the water table" (Sanders and Hers, 2006).

For structure #73, scientists observed the attenuation factors between the ground water and the indoor air of

- $< 1.5 \times 10^{-5}$ for ethylbenzene
- $< 6 \times 10^{-6}$ for xylene and benzene
- $< 1.2 \times 10^{-6}$ for toluene

When compared to the simulated attenuation factors, the actual values were found to be one to two orders of magnitude lower. This suggested that degradation was occurring in the vadose zone. They observed that the

value for MTBE attenuation, (1.2×10^{-5}), was higher than those for the BTEX compounds. "However, it was still in the lower 25th percentile of observed values reported by the U.S. EPA and considerably less than the EPA's generic attenuation factor (1×10^{-3}), despite the presence of sand soil and a short transport distance to ground water" (Sanders and Hers, 2006).

The researchers found soil vapor attenuation factors for benzene and toluene in building #73 to be $< 10^{-5}$, compared to the U.S. EPA generic attenuation factor for nondegradable compounds of 1×10^{-2} . For this structure the researchers also found the following:

- soil vapor attenuation factors for MTBE, 2,2,4-trimethylpentane, and cyclohexane, between 3.6×10^{-4} and 2.2×10^{-5}
- basement and slab attenuation factors, between 7.2×10^{-3} to 8.4×10^{-3} for cyclohexane, 2,2,4-trimethylpentane, and MTBE
- ground floor and basement attenuation factors, between 0.2 and 0.4 for cyclohexane, MTBE and 2,2,4-trimethylpentane

The researchers noted, "The relatively low depressurization observed for building #73 may have reduced soil-gas entry and thus the measured attenuation factor" (Sanders and Hers, 2006).

Conclusions

Sanders and Hers (2006) found that chemical vapor intrusion from gasoline-contaminated ground water was present in area homes, with measurable levels of BTEX chemicals found indoors of all the buildings studied. However, only one of those buildings, #73, "was found to exhibit indoor air impacts attributable to the gasoline, and these impacts were limited to MTBE, 2,2,4-trimethylpentane, and cyclohexane." They determined this based upon the following evidence:

- vapor attenuation patterns between the contaminant

source and the indoor air

- observed indoor concentrations significantly higher than background indoor or outdoor levels or in adjacent buildings
- no observed indoor gasoline sources
- "elevated indoor levels of the aforementioned three chemicals relative to the BTEX chemicals"

The researchers concluded that MTBE is "a potential indoor air contaminant from gasoline-contaminated ground water, although only at high milligrams per liter levels. Measured attenuation factors for all chemicals studied at Stafford Township were one to three orders of magnitude lower than U.S. EPA default factors, even though the site exhibited conditions favorable to vapor intrusion. Finally, while interpretation of measured indoor BTEX concentrations is often difficult due to multiple possible sources, the analysis of tracer chemicals associated mainly with gasoline (MTBE and 2,2,4-trimethylpentane) can help confirm indoor impacts from this contaminant" (Sanders and Hers, 2006).

Reference

Sanders, P.F. and I. Hers, "Vapor intrusion in homes over gasoline-contaminated ground water in Stafford, New Jersey," *Ground Water Monitoring & Remediation*, Vol. 26, No.1, 2006; www.ngwa.org

UTTU thanks Dr. Paul Sanders, paul.sanders@dep.state.nj.us, for his help on this article.



Evaporation and biodegradation in Antarctica

Researchers investigated fuel spill remediation in Antarctica. The researchers sought to "quantitatively relate the evaporation indices to the amount of mass loss using a simple, inexpensive methodology that

can be used on site in remote regions as part of a monitoring and validation process." They used gas chromatography with flame ionization detection (GC-FID). They developed an evaporation model that could be applied at low temperatures to correlate fractionation with mass loss (Snape and others, 2005).

In a continuation of this research, Snape and others (2006) also investigated natural attenuation at three sites within Casey Station, located on the east coast of Antarctica. The sites examined included the Old Casey workshop, the New Casey main powerhouse, and the Old Casey waste disposal site in the Thala Valley. The Old Casey workshop was contaminated in 1982 when 36,000 L of fuel leaked during fuel transfer. The New Casey main powerhouse was contaminated in 1999 when approximately 2000 L of fuel leaked from a storage tank. The Old Casey waste disposal site was heavily contaminated by leaking buried fuel drums, and the contamination continued despite partial remediation from 1995 to 1996.

Methods

Researchers reported that, though a wide variety of petroleum products are used in Antarctica, the most common is Special Antarctic Blend (SAB). This blend is also the most frequently spilled fuel type. Thus, this study focuses upon the evaporation and degradation of SAB though it should also work for other diesel-range organics. SAB is primarily comprised of alkanes in the range $n\text{-C}_{9-14}$ with trace amounts of $n\text{-C}_{15-23}$ also present.

Quantifying mass losses by evaporation

The researchers developed a theoretical model of fuel component fractionation in the Antarctic environment. The model approximated the direct evaporation of a surface spill without allowance for "chemical gradients associated with boundary layer effects, sorption or soil covering." It was based on published vapor pressure

and GC-FID data (Snape and others, 2005). The researchers divided fuel into categories including the following:

- *n*-alkanes
- acyclic isoprenoids
- naphthalene
- methyl-naphthalenes

Also considered in the model were temperature, effective carbon number, vapor pressure, total molar amount, evaporation ratios, and the vapor pressure of a fuel component. The researchers noted limitations to this design due to the small amount of vapor pressure data at low temperatures. In addition, "there is a large temperature difference between GC analysis and the Antarctic environment, and because of differential column retardation of different classes of compounds" (Snape and others, 2005).

The researchers assessed the model by running two evaporation experiments on SAB, one at 20°C and the other at -20°C. They separated portions of SAB into thirty 40 mL vials. Initial mass was taken before the fuel was slowly evaporated, with vials reweighed at various stages of evaporation and mass noted. They found that evaporation rates varied significantly between the temperatures. At 20°C, more than 99% of the fuel evaporated from the vials in 3 days, while after 10 days only 45% of the fuel at -20°C had evaporated.

GC analysis

Scientists extracted samples with hexane. They conducted analysis on a Varian 3800 fitted with FID. "Samples (1 µL) were introduced, either split (20:1) or splitless, with a 280°C injector temperature. These were cross-calibrated with an in-house SAB standard. Initial oven temperature was 35°C, which was held for several minutes followed by a ramp to 310°C at 10°C/min. The FID was heated to 300°C. Combined areas

under resolved peaks and the unresolved complex mixture (UCM) were integrated relative to internal standards. Observed fractionation trends were then compared with predicted evaporation model results" (Snape and others, 2005).

Biodegradation investigation

The scientists conducted microcosm experiments to track changes in SAB as biodegradation progressed at 4°C. They isolated four hydrocarbon-degrading bacteria from the soil microcosms and identified all as *Pseudomonas* spp. These bacteria were then used in the low-temperature biodegradation experiment. This experiment involved the following materials:

- 250 mL screw capped amber glass bottles with Teflon septa
- 170 mL Bushnell-Haas mineral salt broth
- squalane and adamantane

The bottles were autoclaved at 121°C for 20 minutes, cooled, and 250 µL of a filter-sterilized SAB standard with squalane and adamantane were added. Squalane and adamantane were used to gauge abiotic losses from the microcosms. Then a psychrotolerant hydrocarbon-degrading soil bacteria was added. Scientists aseptically flushed all microcosms to ensure aerobic metabolism. They rotated the microcosms on their sides at 12 rpm in the dark at 4°C. They determined hydrocarbon disappearance with sacrificial sampling on days 0, 21, 55, 71, 105 and 146.

Natural attenuation investigation

In 1999 Snape and others (2006) collected soil samples from each site at depths of 3 to 5 cm and 1 m. The samples were stored in glass jars and frozen until analysis at 18°C. Analysis consisted of the extraction and GC-FID methods described above.

Results

Evaporation

The scientists found that "the evaporation model predicted fractionation of the chosen ratios for a specified fuel and temperature." Evaporative losses were relative to the initial starting composition of the reference fuel. The scientists observed fuel component loss in order of GC elution with no preferential losses between *n*-alkane peaks and other components with similar retention time. "Although there were large differences in evaporation rates between the 20 and -20°C experiments, differences in fractionation of the various compounds were small and generally consistent with predicted model results" (Snape and others, 2005).

Snape and others (2005) reported that the theoretical model and observed data had very good agreement for the 20°C experiment, and that fractionation was more selective at -20°C "but the agreement between the experiment and model was not as good." The model gave correct form of the function but was biased toward higher fractionation, likely due to limited vapor pressure data. They predicted experimental data of mass loss to be within ±5%.

Biodegradation results

Scientists first observed biodegradation after 21 days "when naphthalene, the methyl-naphthalenes, and *n*-C₉ were clearly degraded relative to the controls." After 55 days, naphthalene, methyl-naphthalenes and *n*-C₉ were indistinguishable from the UCM." Di-methyl-naphthalene was not degraded. They observed significant degradation in the following:

- *n*-C₁₀ and *n*-C₁₁, by day 55 (*n*-C₁₀ ~60% degraded)
- *n*-C₁₂ and *n*-C₁₃ (all flasks), *n*-C₁₄ (some flasks), by day 146

The scientists reported that "ratios such as $n\text{-C}_{11}/R + \text{UCM}_{\text{ECN}10.5-11.5}$, $n\text{-C}_{12}/i\text{-C}_{13}$ and $n\text{-C}_{13}/i\text{-C}_{14}$ clearly decrease in comparison with abiotic controls. Importantly, $i\text{-C}_{13}$, $i\text{-C}_{14}$, $i\text{-C}_{15}$ and the UCM in the range $n\text{-C}_{9-12}$ did not change significantly throughout the experiment, while the n -alkane:isoprenoid and n -alkane:UCM ratios of similar volatilities changed significantly as biodegradation proceeded." When the microcosms were compared, they discovered that major changes in chemical ratios are due to biological origins. In addition, they determined that total mass losses are primarily caused by biomineralization of light alkanes (Snape and others, 2005).

Natural attenuation results

For the Old Casey workshop, soils were highly contaminated with fuel. The contamination was persistent to depths of at least 80 cm at concentrations of approximately 4000 mg fuel kg^{-1} . Subsurface waters varied in hydrocarbon concentration from approximately 2000 to 92,000 μg fuel L^{-1} . They determined that biodegradation had not occurred and that the $n\text{-C}_{10-12}$ was modified only by evaporation. Evaporation losses included the following:

- > 25% of $n\text{-C}_{10}/i\text{-C}_{14}$
- 40 to 55% of $n\text{-C}_{11}/i\text{-C}_{14}$ and $n\text{-C}_{12}/i\text{-C}_{14}$

The researchers also found little difference in $n\text{-C}_{10-12}$ relative to $i\text{-C}_{14}$ (or $i\text{-C}_{13}/i\text{-C}_{16}$) between samples near the surface and depths of 40 or 80 cm.

For the New Casey Station main powerhouse, Snape and others (2006) observed high levels of contamination in soil samples, 92,500 mg fuel kg^{-1} near the surface, and in water samples at concentrations as high as 8900 μg L^{-1} . Evaporation was determined to be "a significant mechanism of fuel loss," at 45 to 55% by mass. They found only one sample to have fractionated, indicating biodegradation, "with $n\text{-C}_{15}/i\text{-C}_{16}$, $n\text{-C}_{17}/pr$, $n\text{-C}_{18}/ph$ 50%, 63% and

31% lower respectively. Such changes are consistent with minor stages of biodegradation and are probably most obvious because overall contaminant levels in this sample are sufficiently low" (Snape and others, 2006). The soil samples at the Thala Valley waste disposal site were found to have considerably lower fuel concentrations than the other two sites. Virtually no evaporative loss was found.

Conclusions

Snape and others (2005) concluded that their theoretical model and experimental results "clearly identified which ratios change most at various stages of evaporation." Knowledge of initial fuel composition can allow fractionation of compounds with different volatilities to be ascribed to evaporation quantitatively and mass loss to biodegradation. They noted, "to illustrate the relationship between evaporation/biodegradation indices and mass loss for diesel range fuels, it is useful to consider degradation in four stages—defined here as minor (0–25%), intermediate (25–50%), major (50–75%) and extensive (75–100%) losses."

They reported that a poor understanding of fuel partitioning in cold regions was a limiting factor. In addition, they noted that water washing was not considered in this study. "However, subtle changes in the unidentified resolved peaks and the UCM should be considered where extensive water washing is suspected. In these sites the selective removal of relatively soluble compounds such as aromatics will change the shape of the UCM to some extent. In practice, we found that not having reliable reference data for the fuels was more of a limitation to accurately tracking the degradation pathways of old spills. Even a specialized product like SAB has varied significantly over time, and care should be taken when extrapolating parameters derived from modern reference fuels to old

spills" (Snape and others, 2005).

The researchers reported that their results would be useful for tracking bioremediation progress and could be applied to any bulk composition in the diesel range of compounds. They concluded, "although other more penetrative techniques and chemical markers could be used to monitor degradation progress, using a relatively inexpensive GC-FID on-site in remote cold regions like Antarctica offers an affordable validation tool that provides significantly more information regarding degradation pathways than simply reporting losses of total petroleum hydrocarbons" (Snape and others, 2005).

For the natural attenuation research, Snape and others (2006) concluded that, for the Old Casey workshop site and the New Casey main powerhouse site, intermediate evaporative loss "would constrain natural attenuation of these plumes to decades to centuries before concentrations were reduced to < 500 mg fuel kg^{-1} ." The Thala Valley waste disposal site was considered remediated in part by dilution, as over time, contamination hotspots spread in the soil, lowering soil sample concentrations. The researchers concluded that winter spills were characterized by "significant amounts of evaporation in the first few months before the ground thawed and infiltration occurred. However, once in the soil, natural attenuation was not a sufficiently rapid process to prevent off-site migration to environmentally sensitive areas, and was not a suitable strategy to manage fuel spills in this coastal Antarctic region."

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UTTU thanks Dr. Ian Snape, ian.snape@aad.gov.au, for his help on this article.



Research notes

Full-scale removal of DNAPL constituents using steam-enhanced extraction and electrical-resistance heating

Heron, G., Carroll, S. and G. Nielsen, *Ground Water Monitoring & Remediation*, Vol. 25, No. 4, 2005; www.ngwa.org

Scientists presented their results from a "full-scale remediation for DNAPL-impacted soil and ground water using steam-enhanced extraction and electrical-resistance heating in combination. The heating techniques were combined with a robust vapor and liquid extraction strategy that ensured that hydraulic and pneumatic control was maintained during remediation, preventing spreading of the mobilized contaminants" (Heron and others, 2005).

The contaminated site, located in Largo, Florida, was the former Department of Energy (DOE) Pinellas Plant, now the Young-Rainey STAR Center. Eastern portions of the site were used for waste and construction disposal during the 1960s. During the mid-1980s, contamination was detected west of the disposal site. In 1992 an interim ground water recovery system was installed, which was run until 1997, when a larger, nine-extraction-well, pump-and-treat system was installed. In 2002, the

site was approved for an Interim Measures Work Plan for Remediation of NAPL.

The test area consisted of the following:

- 15 steam injection wells
- 28 extraction wells with electrodes
- 21 combined steam injection-electrode wells
- two deep electrodes
- 36 temperature-monitoring boreholes
- four pairs of ground water monitoring wells

The scientists applied steam at high rates to the surficial aquifer, while constantly monitoring subsurface temperatures. Liquids extracted by the 28 extraction wells were cooled, condensed, and separated in an on-site treatment system. The NAPL was separated from the water and the water was screened for volatile contaminants.

Heron and others (2005) determined that combining steam delivery to permeable zones and electrical heating to low-permeable zones was an effective mode of uniform heating. Heating that focused on the perimeter of the treatment site effectively created a DNAPL-containing volume, which reduced horizontal and vertical contaminant spreading risk. They also determined pressure cycling to be an important factor in accelerating mass removal and shortening operational time. "During the induced pressure cycles, mass removal rates were many times higher than during constant injection and extraction of steam and electrical power."

The scientists concluded that the removal of approximately 2500 lb of volatile contaminants in 4.5 months reduced the contaminant concentrations in soil and ground water to a point that was "believed to be at least 100 times lower than levels that would allow DNAPL to exist." Ground water samples taken in three different rounds documented "that rebound did not occur within the 6 months of sampling and showed

that ground water concentrations were lowered to below MCL in the majority of the samples. By such effective remediation estimated at an average mass removal efficacy of 99.9% for the volatile contaminants, polishing steps may not be necessary" (Heron and others, 2005).

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Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degradation: Distribution of the genes in BTEX degrading isolates and in subsurface soils of BTEX contaminated industrial site

Hendrickx, B., Junca, H., Vosahlova, J., Lindner, A., Ruegg, I., Bucheli-Witschel, M., Faber, F., Egli, T., Mau, M., Schlomann, M., Brennerova, M., Brenner, V., Pieper, D.H., Top, E.M., Dejonghe, W., Bastiaens, L. and D. Springael, *Journal of Microbiological Methods*, Vol. 64, No. 2, 2006; www.sciencedirect.com

Scientists investigated primer sets for polymerase chain reaction (PCR) detection of the gene sequences involved in initial attack of bacterial aerobic benzene, toluene, ethylbenzene and xylenes (BTEX) degradation. By "demonstrating the occurrence of catabolic genotypes involved in BTEX degradation or their corresponding mRNA in the aquifer" using sensitive PCR and reverse transcription polymerase chain reaction (RT-PCR) detection methods," BTEX biodegrading bacteria and their activity in the environment may be quickly identified (Hendrickx and others, 2006).

Hendrickx and others (2006) used 12 different bacterial BTEX degrading reference strains for the study. They used soil samples taken in March 2001 at an oil refinery in the Czech Republic that had been

contaminated with BTEX by a ground water plume. Bacterial strains were then isolated from the soil samples and their total genomic DNA extracted. The scientists performed PCR amplification and pattern analysis of the DNA. "Novel generate and degenerate primers were designed based on protein and DNA sequence alignments of the appropriate proteins/genes" using a protein and DNA analysis computer program. The scientists performed PCR amplification, analyzed by electrophoresis. The amplicons resulting from PCR with the different primer sets were purified, the BTEX catabolic gene sequences were sequenced double stranded, and a similarity analysis of the DNA was performed.

Hendrickx and others (2006) reported that benzene degradation was the most common BTEX degradation phenotype "and its distribution was largely congruent with the distribution of the *tmoA*-like genotype. The presence of *tmoA* and *xylM* genes in phylogenetically distant strains indicated the occurrence of horizontal transfer of BTEX catabolic genes in the aquifer. Overall, these results show spatial variation in the composition of the BTEX degradation genes and hence in the type of BTEX degradation activity and pathway, at the examined site. They indicate that bacteria carrying specific pathways and primarily carrying *tmoA/xylM/xylE1* genotypes, are being selected upon BTEX contamination."

The researchers found that eight of the 12 novel primer sets were successful for "PCR detection of gene sequences encoding for mono-oxygenases and dioxygenase enzyme proteins involved in the initial attack of BTEX and encoding for catechol 2,3-dioxygenase involved in the aromatic ring *meta*-cleavage." They concluded that their results "indicate a primary role of these sequences in adaptation of the community to the BTEX contamination and in in situ

degradation of BTEX at the examined site" (Hendrickx and others, 2006).