Research Articles

Bioremediation of PAH Polluted Soils: Column Studies

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Abstract

Background. Due to spills, discharges and leakage, the gaswork site at Husarviken in Stockholm is today the largest (36 ha) creosote-contaminated site in Sweden. The main pollutants are creosote, lead and mercury. The remediation costs are estimated to be as high as US $125 million. It is thus of great interest to find more cost effective remediation methods. **Objectives.** The aim of this study was to investigate i) if the addition of NTA, EDTA, nitrate, iron and dry yeast would enhance the bioremediation rate of a complex organic pollutant like PAH and, if so, at what concentrations they would be most efficient, ii) the effect on PAH reduction when larger dimensions of the column is used to diminish the effect of water passing along the sides of the column, iii) long-term effects on the reduction of PAH in field-contaminated soil with high concentrations.

Materials and Methods. Creosote-contaminated soil from the Husarviken gaswork site was treated with aerated water in column experiments at room temperature. Three column experiments were performed in 2 and 100 L of homogenised soil samples percolated by recirculating flushing water. Fluoranthene was analysed as a representative of the overall degradation of PAH in the columns.

Results and Discussion. The PAH concentration (total 16 Priority USEPA PAH) was reduced from 129 mg/kg to at most 33 mg/kg in the 2-litre columns. A total of four PAH in the soil were reduced from 1330 mg/kg to about 400 mg/kg in the 100-litre columns. Generally, a 70% reduction of PAH concentration can be achieved by bioremediation technology. The transformation and/or degradation of fluoranthene were fast at the beginning of the experiment and then gradually slowed down. This mirrors the impact of the bioavailable fluoranthene, which is initially large, but reaches zero after 200 days.

Conclusions. A simulation model using the fluoranthene data shows that the effectiveness of PAH degradation is, above all, dependent on the bioavailability of PAH. A reduction of 70% of PAH in the soil is applicable to soil containing <200 mg/kg to meet the Swedish recommendations of 60 mg/kg. At Husarviken, soil with <200 mg/kg corresponds to 80% of the polluted area.

Keywords: Bioavailability; biodegradation; polycyclic aromatic hydrocarbon; column experiment; modelling

1 Background

Polyaromatic hydrocarbons (PAH) consist of two or more benzene rings. They are found in high concentrations at many industrial sites and are of environmental concern because most of them are potential carcinogens and mutagens. The most common measures to treat the polluted site are excavation, thermal and/or physico-chemical techniques. These techniques are all associated with high expenditure. This has led to an increased interest in bioremediation, whereby microorganisms degrade the hazardous organic material. The *in situ* bioremediation technique leaves the soil undestroyed and that at a cost which is far less expensive than other methods. The effectiveness of the bioremediation, however, is usually unsatisfactory and requires a good knowledge of the mechanisms of degradation and the factors controlling it [1]. The role of thumb is that the fewer the benzene rings in the PAH molecule, the faster is the degradation [2,3,4]. *In situ* treatment of contaminated soil has been reviewed by a number of researchers [5,6]. For example, the pump and treat method is employed and groundwater recirculated through the soil. The groundwater is usually aerated and/or given some nutrients before recirculation. At the Husarviken gaswork site in Stockholm, Sweden, PAH are found in the uppermost 4 m of the soil, which is unsaturated down to 5 m. The water recharge percolating through the soil is thus well aerated and microbiological degradation of PAH can be considered as primarily aerobic. The general aerobic pathway for bacterial degradation of PAH starts with hydroxylation, involving the incorporation of molecular oxygen [7]. The cis-dihydrodiols formed are dehydrogenated and they rearomatise the benzene nucleus to form dihydroxylated intermediates (catechols).

One factor, usually considered as the limiting factor for most bioremediation technique, is the bioavailabilty of the organic compounds [8]. PAH compounds have a very low solubility in water and they seem to be available for microorganisms only when they are dissolved in water [9,10]. Dissolved PAH, however, sorb to mineral surfaces and need to be desorbed to become bioavailable [11,12,13,14,15]. On the other hand, a study [16] showed that phenanthrene sorbed to mineral-associated humic acids stimulated its mineralisation. The addition of surfactants can also enhance the degradation of PAH, e.g. [15]. The mixture of PAH may also be of importance. McNally et al. [17] reported that the presence of a 2-ringed PAH stimulated degradation of a 3-ringed PAH five-fold and a 4-ringed PAH degradation two-fold. They also reported that the presence of 3-ringed PAH inhibited the degradation of 4-ringed compounds. Biodegradation is hampered for two major reasons i) the compound's less ubiquity compared to natural compounds, and ii) shortage of elec-

tron acceptors available to the microflora [18,19,20,21]. The most efficient electron acceptor is oxygen. With a deficiency of oxygen, nitrate and Fe(III) can serve as electron acceptors. The latter is usually present at sufficient concentrations, but not bioavailable because it is present as a solid. To overcome this drawback, Lovley and his co-workers [20,21] showed that, by addition of NTA (nitrilotriacetic acid), it stimulated the oxidation of organic matter and the reduction of Fe(III). Lovley and Woodward [22] also showed that NTA did not stimulate Fe(III) reduction by acting as a carbon source, and that there was no Fe(III) reduction with or without NTA in the absence of bacteria *Geobacter metallireducens*.

The gaswork site at Husarviken is situated a few kilometres northeast of central Stockholm and was built in 1893. Over the last 79 years (until 1972) it produced 7 billion m^3 of gas from 20 million tons of coal. A large amount of by-products was also produced including 700,000 tons of tar, 13 million tons of coke and more than 100,000 tons of benzene products. Due to spills, discharge and leakage, the gaswork site is today the largest (36 ha) creosote-contaminated site in Sweden. The main pollutants are creosote, lead and mercury. The remediation costs are estimated to be as high as US $125 million.

2 Objectives

The aim of this study was to investigate i) if addition of NTA, EDTA, nitrate, iron and dry yeast would enhance the bioremediation rate of a complex organic pollutant like PAH and, if so, at what concentrations they would be most efficient, ii) the effect on PAH reduction when larger dimensions of the column is used to diminish the effect of water passing along the sides of the column and iii) long-term effects on the reduction of PAH in field-contaminated soil with high concentrations.

3 Materials and Methods

3.1 Bioremediation experiments

Creosote-polluted soil was collected from the gaswork site at Husarviken. In order to avoid a large variation of PAH concentrations between separate columns in the experiment, the soil was homogenised by tumbling in a cement-mixing jar for 30 min, and stones larger than 2 cm were removed. Duplicate columns were used in all cases and the experiments were conducted at room temperature (20°C). Our first attempt in the field would be to enhance the bioremediation procedure through recirculation of treated ground water in the unsaturated zone. The treatment studies in the laboratory have thus been performed with unsaturated soil columns under aerobic conditions to simulate the natural conditions at the Husarviken site.

3.1.1 Short-term column experiment

I. The first experiment was performed for 97 days. 10 columns were constructed of PVC pipes with an inner diameter of 10 cm and a length of 30 cm. Each column was filled with 2 L of soil. The PAH concentration of the original soil was 129 mg/kg. For each column, a 2-litre water container was supplied and the water was pumped to the top of the column. NTA (nitrilotriacetic acid) (1 mM), EDTA (1 mM), NO_3 (0.43 mM), Fe (1 mM) and 0.2 w% dry yeast were added as shown in Table 1. The flow rate was set to the maximum possible limit, which was 9 mL/h due to limitations imposed by permeability. The system was open to air.

II. The second experiment lasted for 121 days. The same columns and water flow as in the first experiment were used, but the containers were 5 L in size. Column tops and containers were covered to prevent evaporation. Before starting the experiment the pH of the circulating water was adjusted to 6.2, which was the original pH of the soil.

3.1.2 Long-term column experiment

The two columns were made of PVC, with a diameter of 40 cm and a length of 1.5 m. Each column was filled with 100 L of the wet homogenised, contaminated soil and connected to a 100-litre water container. 1 L of dry soil weighed 0.83 kg. The PAH concentration in the dry soil fraction <5 mm is 2,000 mg/kg (total 16 Priority USEPA PAH). The <5 mm fraction made up 44 % of the dry soil, i.e. 365 g/L. The total amount of PAH is 73 g in each column. pH varied between 7.7 and 6.8 with the higher values being observed at the beginning of the experiment. Aerated water was recycled to the top of the soil column with a flow rate of 90 mL/h from each container. The retention time of the water in the column was about 60 h and the volume of water in the soil was about 5.5 L. The PAH degradation was monitored by analysing soil samples at the beginning and at the end (after 800 days) of the experiment and by analysing fluoranthene in the recirculated water on several occasions during the experiment. To prevent evaporation, the columns were sealed at the top. To prevent and monitor degassing of PAH from the water, the containers were covered, and the outgoing air passed through a carbon filter. The carbon filters were analysed on two occasions. No PAH were detected during both occasions.

In order to stimulate microbial activity, dry yeast and NTA were initially added to final concentrations of 0.04% and 1 mM to the water circulating in columns A and B, respectively. Yeast may have a similar effect as NTA, but also contains nutrients and vitamin B that may be stimulating to the indigenous fauna. A second addition was made after 104 days, but in the opposite columns. After 743 days, NTA was added to a final concentration of 1 mM into both containers.

3.2 Analytical procedures

Soil samples were air dried and the <2 mm fraction was ground. A Soxhlet unit was used to extract the PAH using 3 g of dry sediment with 120 mL toluene for 18hr. The extract was purified with a silica column (10 g deactivated silica with 10% water). PAH was eluated with 25 mL hexane followed

by 25 mL hexane:dichlormethane (3:2 v/v). Analysis was performed on a GC-MS (GC: Fisons GC 8000; MS: Fisons MD 800, EI-ionisation). The column was a 60-m DB-5, 0.32 mm ID and 0.25 µm film (J&W Scientific, CA). The carrier gas was He. Injection temperature was 250°C and the temperature programme was: 80°C for 2 min, 8°C/min to 300°C and then kept at 300°C for 20 min. All concentrations reported are extractable concentrations and not necessarily total concentrations. There may be a reduced recovery of PAH due to weathering and ageing [23].

In all column experiments, water samples were manually extracted by a solid phase microextraction (SPME) technique, using a 7 µm polydimethylsiloxane-coated fibre from Supelco. The SPME fibre was exposed in the water sample for 10 minutes and stirred at 20°C. The fibre was injected directly on a GC-MS system via a special syringe. This is a rapid, cheap and simple screening method for analysing PAH in headspace and water [24,25,26]. Calibration checks in these experiments showed that the standard deviation for fluoranthene analysis in water was 6%.

3.3 Stable isotopes

The $^{13}C/^{12}C$ ratio is reported as $\delta^{13}C$ values in ‰ relative to the international reference standard PDB (*Belemnitella americana* from the Cretaceous Peedee formation, South Carolina, US). Analyses were made on freeze dried samples.

3.4 Modelling procedure

The experimental data was used for simulation using the software ModelMaker 3.0 (http://www.cherwell.com). First-order kinetics was used for the simulation of transfer between compartments. The rate law for a first-order reaction is

$$-d(A)/dt = k(A)$$

where k is the kinetic constant. At time t = 0, the concentration A is A_0 and at a later time t it is A_t. One can argue that such simulation is dependent on the initial concentration, especially when it is coupled to low concentrations [27]. In our case, however, we are dealing with high concentrations. The time-step is one day. Results are presented as graphs and simulations were optimised based on least-squares minimization using the Levenberg-Marquardt algorithm. This is a X^2 (chi squared) measure, defined as

$$X^2 = \sum \frac{(m_i - o_i)^2}{\varepsilon_i^2}$$

where

o_i is the value of the ith observation
e_i is the error estimate for that observation, and
m_i is the model prediction for that observation

4 Results and Discussion

4.1 PAH degradation

The experiments were conducted at 20°C. The reaction rates are therefore expected to be somewhat higher than those at natural conditions where the temperature of the ground water is 5–10°C during April–December. As the experiment took place under aerobic conditions there was always a sufficient amount of oxygen to act as an electron acceptor. At the Husarviken site, the lowest oxygen concentration recorded was 1 mg/L.

4.1.1 Short-term experiments

(1) Final results are depicted in **Fig. 1a**. In the controls, an overall reduction of PAH concentrations by 15% was achieved. Addition of nitrate had only a slight effect (36%) on total PAH reduction compared to the controls. Nitrate is probably not improving the PAH reduction by acting as an electron acceptor, but only for the lower part of the column where the aerobic conditions may deteriorate. The addition of 1 mM NTA enhanced reduction to 67% of the original concentration, which is somewhat better than with EDTA (61%). In separate experiments, we found that EDTA was resistant to transformation and remained in solution. NTA, on the other hand, was either adsorbed to the soil particles and/or degraded as it disappeared from solutions within 3 weeks. Thus, from an environmental point of view, it is less harmful to use NTA than EDTA. The addition of iron did not improve PAH reduction compared to the sole addition of NTA.

(2) Final results are depicted in **Fig. 1b**. The controls show a 36% reduction of PAH concentration. This is significantly better compared with the previous experiment and may be a result of a more active indigenous fauna and/or a larger amount of bioavailable PAH. The best result (69%) with NTA was achieved with a concentration of 4 mM. Improvements in PAH reduction could not be achieved with NTA concentrations >4 mM. We can speculate on one of the reasons, whereby concentrations of toxic metals like Pb and Cu are chelated, and increase due to greater solubility, and are substantially higher compared to normal levels in soil. However, the best overall result (74% reduction) was achieved with dry yeast. Dry yeast may thus stimulate the bioactivity by its content of nutrients and vitamin B. We have no explanation for the relatively large degradation of 5+6 ring PAH, which is in conflict with published data where according to the literature the 2+3 ring PAH should exhibit the highest reduction [2,3,4]. Thus, an addition of 1–4 mM NTA would be sufficient to stimulate the bioremediation of PAH in the Husarviken soil. Addition of iron had no effect on PAH reduction. This is probably expected as the soil is also 'polluted' with iron cyanide. The total iron concentration in 5M HNO_3 leached soil is 26 g/kg.

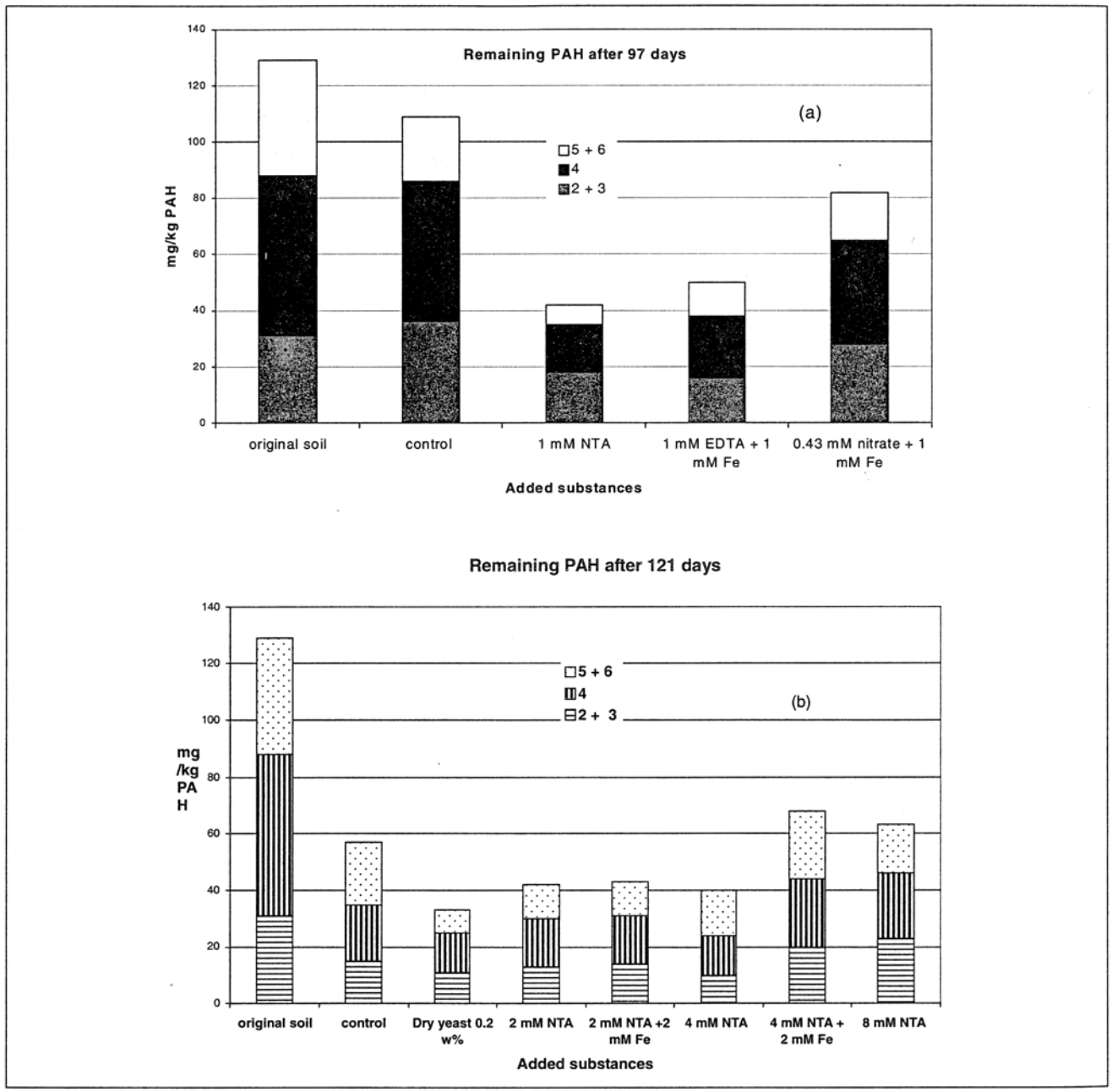

Fig. 1 a,b: Remaining PAH in the 2 litre column experiments after 97 (a) and 121 (b) days, respectively. The PAH is divided into 2+3, 4 and 5+6 rings

The short-term experiments showed that the degradation increased from about 20% in the control to about 60% in the columns with additions of NTA. The stimulation effect by NTA is caused by complexation of Fe(III) [20,22].

4.1.2 Long-term column experiment

The total concentration of PAH is 73 g in each column out of which 49 g represents the four PAH listed in **Table 1**. The amount of these four PAH remaining after 800 days was 15 g. Hence, the reduction after 800 days was 34 g PAH, corresponding to 69%.

Table 1: Initial (untreated) and final concentrations of PAH

PAH	Untreated soil (mg/kg)	Column A (mg/kg)	Column B (mg/kg)
Phenanthrene	352	98	87
Anthracene	275	85	90
Fluoranthene	463	111	123
Pyrene	240	110	96
Total	1330	404	396

The results exhibit a good agreement between both columns. Individual concentrations of different PAH were reduced by 50–75%. Pyrene was degraded least during the course of the column experiments. Even though the PAH concentration has been reduced by 69% during 800 days of treatment, the remaining concentration is still too high to meet the 60 mg/kg recommended by the Swedish EPA. A 70% reduction of PAH would thus be applicable to soil containing <200 mg/kg. At Husarviken, this corresponds to 80% of the polluted soil.

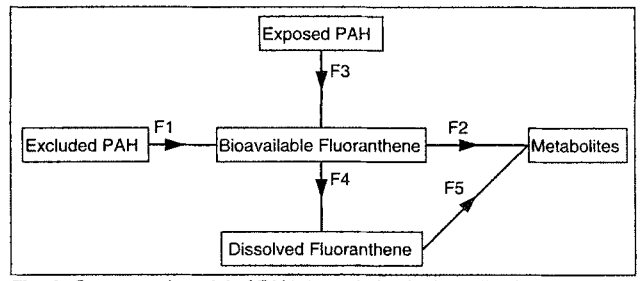

Fig. 2: Conceptual model of PAH degradation in the soil columns

4.2 Stable isotopes

The $\delta^{13}C$ values in the treated soil were slightly higher (–23.50, column A and –23.28, column B) than in the untreated soil (–23.76), and can be interpreted as a biological degradation of the organic material. However, one would have expected the values to be even higher in the treated soil, since microbes prefer light carbon PAH (i.e. ^{12}C-PAH), which are degraded into metabolites and finally carbon dioxide, leaving the heavy PAH (i.e. ^{13}C-PAH) in the soil. This means that ^{12}C is enriched in the metabolites while the remaining PAH will be enriched in ^{13}C, resulting in an increased value of the $\delta^{13}C$ in the soil. It is possible that the end product in this experiment is only to a minor extent carbon dioxide and to a larger extent non-volatile metabolites, which leaves the $\delta^{13}C$ of the bulk organic matter in the soil almost unchanged. The observed decrease in the concentration of fluoranthene is thus merely due to transformation into intermediates rather than complete transformation to CO_2. A similar result has been indicated in a comparative study between a sterilised and non-sterilised soil [28] with the almost complete disappearance of aromatics from the 'live' soil, but limited release of CO_2. The authors suggest that the microbial activity may have generated metabolites such as quinones and hydroxylated or carboxylated intermediates, which are more reactive than unsubstituted molecules. These metabolites are more likely to sorb and become incorporated or bound into the soil organic matter [29]. The $\delta^{13}C$ of the bulk soil organic matter at the end of the experiment would thus exhibit very little change compared to the initial value.

4.3 Modelling of PAH bioremediation in the 100-litre columns

Bioremediation of PAH in soil involves several processes like adsorption/desorption, hydration, diffusion, microbial degradation etc. In a conceptual model (Fig. 2) of the remediation processes we limited the number of rate limiting factors to accessibility, adsorption/desorption and bioavailabilty.

The solid PAH components are probably exposed in the soil to a different extent, but are represented by two major compartments in the model. In the first compartment, Excluded PAH contains excluded or non-labile fluoranthene in the form of clods or enclosed by soil to an extent that only a minor part of it is exposed to the percolating water. The second compartment Exposed PAH contains easily accessible or labile fluoranthene well exposed to the percolating water and the indigenous fauna. Fluoranthene released from these two compartments are transferred to another compartment, Bioavailable Fluoranthene. This fraction represents the amount that is adsorbed to the soil material, but exposed to biodegradation and desorption by the percolating water. The mass transfer from the Exposed PAH compartment has been given a kinetic constant, which is almost two orders of magnitude higher than that from Excluded PAH. This is in agreement with previous studies i.e. Cornelissen et al. [13]. The authors indicated an initial phase of rapid degradation with a rate constant at approximately 10^{-2}–10^{-3} h^{-1}. Subsequently, the degradation rate decreased to 10^{-3}–10^{-4} h^{-1}. The rate constants noted in this study are approximately 10^{-3} h^{-1} and 10^{-5} h^{-1}, respectively, thus in the lower range of those observed by [13]. The final compartments of Dissolved Fluoranthene and Metabolites represent fluoranthene in solution and the metabolites formed during the transformation/degradation process. However, in spite of the good fit between the model graph and the experimental data, the model does not describe the intrinsic chemical properties of the soil or the different types of microbial species that take part in the degradation process. The variation of observed data at the beginning of the experiment is in agreement with our assumption that different fractions of PAH exhibit a variation in exposure to biodegradation and kinetics. When only the non-labile fraction is available during the second half of the experiment, the observed data exhibit a more stable trend. Compartment values and kinetic constants are listed in Table 2.

Table 2: Parameters of optimised computer model. k_i is the kinetic constant of flow F_i

Column	Initial content Excluded PAH (mg)	Initial content Exposed PAH (mg)	k_1 d^{-1}	k_2 d^{-1}	k_3 d^{-1}	k_4 d^{-1}	k_5 d^{-1}	Obs. final PAH cont. (mg)	Est. final PAH cont. (mg)
A	16000	1100	0.0006	0.99	0.03	0.01	1	4100	9870
B	14900	2200	0.0006	0.98	0.03	0.02	1	4550	7750

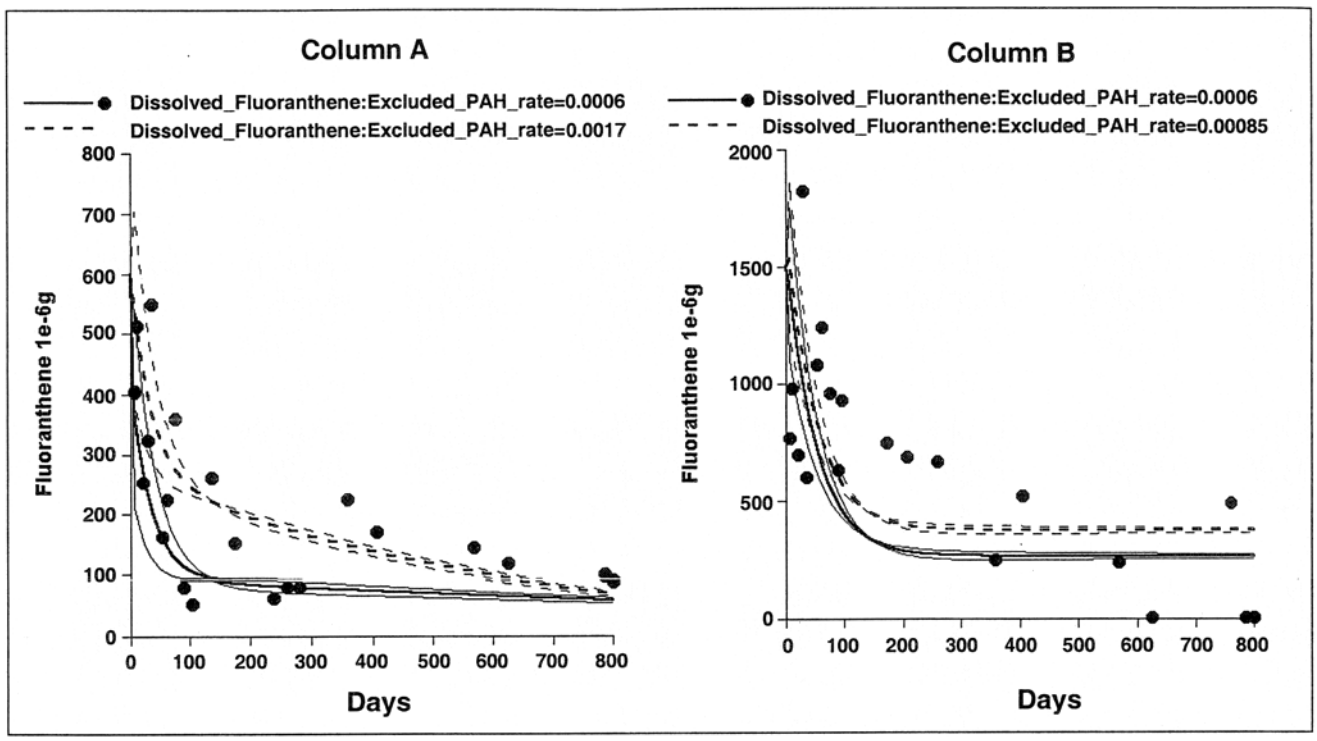

Fig. 3: Total amount of fluoranthene in water percolating through the soil in columns A and B. Symbols represent analytical data and depicted graphs represent computer simulation at two different rate constants of k1. The solid lines represent the rate constants after an optimisation procedure. The broken lines represent the values needed to meet the observed final concentration of fluoranthene. The fine confidence lines represent simulation where the initial amount of Excluded PAH is given a variation of ± 1000 mg, which is affecting the Exposed PAH with the opposite amount

The resulting graphs are depicted in Fig. 3 along with data observed from water samples. These exhibit a general trend with varying, but decreasing, fluoranthene concentrations over time. The concentration of dissolved fluoranthene is higher in column B (500–1800 mg/L) compared to column A (100–500 mg/L), in spite of the fact that they contained the same homogenized soil and the same amount of percolating water. The higher concentration of dissolved fluoranthene in column B could only be represented by a model in which we apply twice the value of Exposed PAH in column A, and a higher rate constant for the transfer from Bioavailable to Dissolved Fluoranthene (F4). Exposed PAH and kinetic constant k4 were the two most sensitive parameters for the simulation of observed fluoranthene data in the model. Initially, Bioavailable Fluoranthene is abundant, but decreases quickly as it is transformed into Dissolved Fluoranthene and Metabolites. It is quite probable that the two columns were packed in such a way that they differed with the amount of PAH that was easily accessible for degradation. Changes in the flow rate of F2 and F3 had a negligible effect on the final result. The rate constants of k3 and k4 were of the same order of magnitude as previously reported mineralisation rates for phenanthrene and chrysene [30]. The differences between the two columns can also be attributed to differences in apparent distribution coefficients (K_d). If this is expressed as the ratio between bioavailable and dissolved fluoranthene, the values of columns A and B are 100 and 50, respectively. At K_d values >10, the water saturation of the soil will have no influence on the detainment of fluoranthene [31].

The transfer of fluoranthene from Dissolved and Bioavailable Fluoranthene to Metabolites is extremely fast and is not a limiting factor in the overall transformation process. The compartment Exposed PAH is emptied in about 200 days while the output from the Excluded PAH takes longer. Thus, the main obstacle in the remediation/degradation process is not the chemical transformation and/or the intrinsic microbial activity per se, but the accessibility of the PAH. Our results are in line with previous studies [32,33,34] that conclude the rate-limiting factor to be the mass transfer of PAH out of pores where it is physically protected from microbial activities.

5 Conclusions

- 1–4 mM NTA has a significantly positive effect on the reduction of PAH in soil. The rate of reduction for PAH is better than with EDTA. Addition of Fe of had no effect.
- The transformation/degradation of fluoranthene was fast at the beginning of the experiment and then slowed down. This mirrors the impact of bioavailable fluoranthene, which is initially large but reaches zero after 200 days.
- Longer treatment time would further decrease the concentration of the remaining PAH, but the reduction process is slowing down rapidly after 400 days. The cause for this may be the strong sorption of PAH to soil particles. To achieve a faster and more effective degradation, it would be necessary to mobilise the remaining recalcitrant PAH by exposing it better to the degradation processes.
- A 70% reduction of PAH concentration can be achieved with minor additions of NTA. To meet the Swedish EPA

recommendations of a maximum of 60 mg/kg the *in situ* bioremediation of PAH is limited to soils with concentrations <200 mg/kg. This is applicable to 80% of the soil at Husarviken.

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