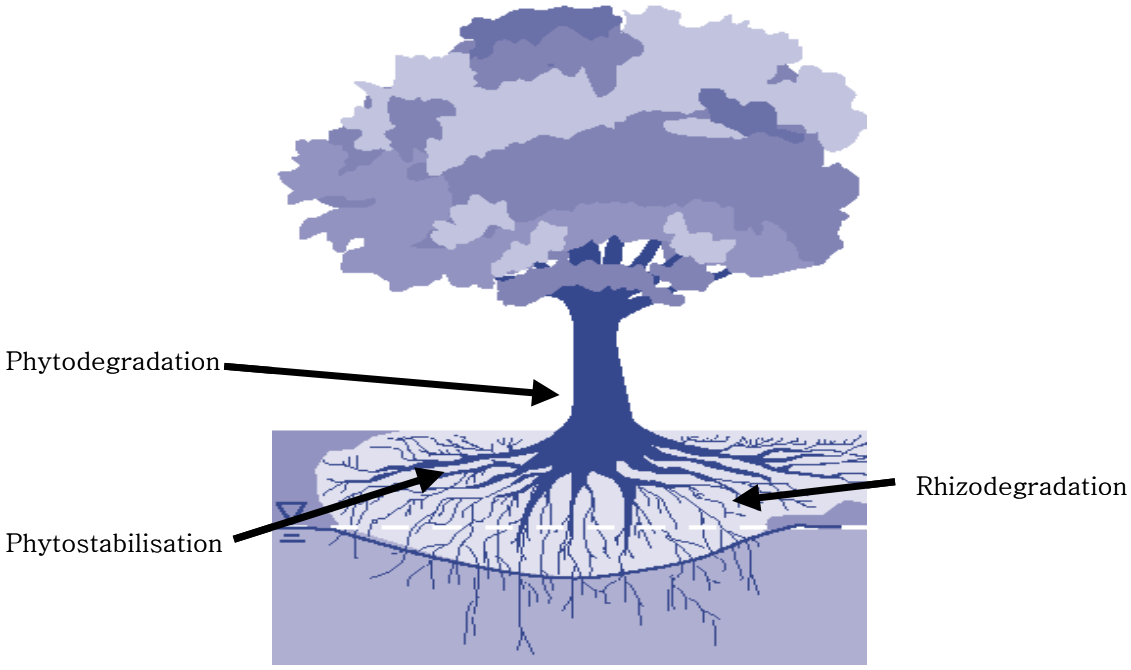

Phytoremediation of Petroleum Hydrocarbon-Contaminated Soil with *Salix viminalis*

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MAXIME HIRTZ

Preface

This thesis was carried out at the Department of Water Environment and Transport, Chalmers University of Technology, Göteborg under the supervision of Professor Greg Morrison. It was also supervised and initiated by Sonja Blom at FB Engineering and carried out with the help of Preem Refinery AB.

We would like to express our gratitude to all those that has helped us throughout the project, especially to Sonja Blom at FB Engineering for her guidance and support and to Greg Morrison that has been a valuable support. We would also like to thank Thomas Wennerberg at Preem who made the analyses possible and the staff at Preem laboratory, especially Katarina Munter, for their help and company. At Chalmers we wish to thank Lars Ove Sörman for his help with the set up of the experiments, Jesper Knutsson and Babar Muhammad for their laboratory assistance and Linda Katzenellenbogen and Henriette Melin for their administrative and technical help.

Abstract

The main objective of this thesis has been to study the efficiency of *Salix viminalis* when remediating petroleum hydrocarbon-contaminated (PHC) soils. The experimental design was a continuous flow system with downward flow and with an input solution consisting of diesel (mainly straight aliphatic chains from C_{12} to C_{16}) dissolved in a solution of 0.5% methanol in water. Plants were continuously fed with a nutrient solution and exposed to the contamination during 24 hours for each experiment. The influence of flow rate and concentration of contaminants on the containment of PHC in the soil was investigated. Analysis of total petroleum hydrocarbons in the affluent was performed by infrared spectrometry (IR). For 7 out of 9 experiments, PHC concentrations in soil were seen to increase and reach equilibrium after about 10 hours of exposure. Saturation of the soil was never obtained. The amount of PHC sorbed into the soil was calculated, from the input and output PHC concentration, through a mass balance equation. A comparison between the concentrations of PHC in soil and water was presented in a sorption curve that was found linear, with a good regression coefficient ($r^2 = 0,93$). From its slope the soil-water partitioning coefficient (K_p) was calculated ($K_p = 36$). Sorption occurred mainly on the organic carbon of the soil. Plants can enhance sorption of PHC in producing mycchoriza and root exudates that increase the content of organic carbon in the soil. This was confirmed by GC-MS analyses of soil samples, which indicates that phytostabilisation increased the containment of petroleum hydrocarbons in the soil. The other phytoremediation processes of PHC-contaminated soil, phytodegradation and rhizodegradation, were only studied in theory.

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1 Introduction

In Sweden pollution of petroleum products is, next to heavy metals, the most common type of soil contamination (Lindmark et al.1995). The pollution have in many cases its origin in leaking above and underground storage tanks and pipelines at gasoline stations, oil refineries, oil storage facilities, industries etc. The pollution of soils and surface- and ground waters by petroleum products leads to ecological and health risk for man and the environment of varying size (Frick et al., 1999).

Phytoremediation, or the use of plants to remediate contaminated soils and water environments, has become an area of intense study. Vegetation has been found to play an important role in the reduction of toxic organic chemicals. For petroleum hydrocarbons (PHC), the presence of plants impacts contaminant biodegradation by providing an optimal environment for microbial production in the root zone. This often leads to enhanced reduction of PHC in soils that are vegetated, compared to nonvegetated soils (Fiorenza, et al., 2000). Contamination can also be reduced as a result of plant uptake into the tissue were it can be further degraded to harmless substances or immobilisation in soil through absorption and accumulation by roots and adsorption onto roots (US EPA, 2000).

Willows (*Salix* spp.) are commonly used in Swedish short-rotation forestry, mainly to produce biofuels. Willow clones have been selected for their high growth potential under Scandinavian climatic conditions (Rytter, 2001). In Sweden studies in phytoremediation has, so far, focused on the ability for plants to accumulate metals, and not so much on the reduction of organic compounds.

This study investigates the possibility to use *Salix viminalis* for enhanced containment and degradation of PHC in soil. The focus of the experimental studies is on the initial part of the phytoremediation process, when PHC reach the soil, and the sorption mechanisms that determine the uptake rate. Furthermore is the ability to degrade PHC in oil-contaminated soil discussed and the mechanisms that controls biodegradation.

2 Background

2.1 Petroleum hydrocarbons-contaminated soil

Petroleum hydrocarbons are naturally occurring chemicals used by humans for a variety of activities, such as the fuelling of vehicles and heating of homes. Natural gas, crude oil, tars and asphalts are types of petroleum hydrocarbons composed of various proportions of alkanes and aromatics.

Leaks and spills of petroleum products is, next to heavy metals, the most common type of soil contamination in Sweden (Lindmark et al.1995). The pollution have in many cases its origin in leaking above and underground storage tanks and pipelines at gasoline stations, oil refineries, oil storage facilities, industries etc. Some of the products, like gasoline, diesel, and fuel oil, can move rapidly through the soil and into ground water. This presence of petroleum in soil and ground water can lead to ecological and health risk for man and the environment of varying size (Frick et al., 1999).

Petroleum products contain numerous potentially toxic compounds, including common solvents (benzene, toluene and xylene) and additives, such as ethylene dibromide (EDB), and organic lead compounds. EDB is carcinogenic (cancer-causing) to laboratory animals and benzene is considered a human carcinogenic compound (Bernes, 1998).

The Swedish reference values for levels in polluted soils for total extractable aliphatic substances and total extractable aromatic substances, which are major constituents in many petroleum products, are 80 and 30 mg/kg of dry substance.

Only in Sweden it is estimated that over 2500-3000 shut down gasoline stations need some sort of remedial action to avoid negative effects on the environment. Furthermore there is need for remedial action at numerous other sites in Sweden, which have been used as depot for other kinds of petroleum products (Larsson et al., 2001).

2.2 Petroleum Hydrocarbon in soil

The fate of PHC in the environment depends on various factors, both physical and chemical. Physical factors plays the most important role for sorption to take place, while the chemical factors primarily determines the degradation processes.

2.2.1 Physical factors

- Solubility
  Since biological degradation is taking place in the water phase, the more hydrophilic PHC are the more easily biodegraded are they. On the contrary, the more hydrophobic PHC are they are more sorbed onto the organic matter in the soil.

- Permeability
  The greater the permeability, the easier it is to distribute nutrients and an electron acceptors to the PHC-contaminated solids and ground water. These conditions also tend to lead to greater extent of contamination.

- Soil type
  In addition to permeability, soil type also impacts the degree of adsorption of contaminants and nutrients by the soil. Sand and gravel are the most favourable soil types for transport; clays are the least favourable. The soil organic matter content (e.g., humates) impacts the movement of petroleum hydrocarbons through the aquifer.

- Soil moisture
  Soil moisture is essential to biodegradation since the majority of microorganisms live in the water film surrounding soil particles. Soil water serves as the transport medium through which many nutrients and organic constituents diffuse to the microbial cell, and through which metabolic waste products are removed. Soil moisture content in the range of 50-80% is optimal for biodegradation.

- Soil temperature
  Soil temperature is a controlling factor for the rate of biodegradation. Higher soil temperatures result in higher microbial metabolic activity and higher rates of biodegradation. Biodegradation essentially stops at 0°C. Most reported biodegradation rates have been determined at 20°-25°C (Fiorenza et al., 2001).

2.2.2 Chemical Factors

- Oxygen
  A large fraction of the microbial population within soil depends on oxygen as the terminal electron acceptor in metabolism, and the rate of aerobic biodegradation is typically limited by the rate at which oxygen is supplied. The solubility of oxygen in water is low at best; about 10 mg/l at 25°C (Lindmark, et al., 1995).

- Soil pH
  Soil pH is an indicator of hydrogen ion activity in the soil. A pH in the range of 5 to 9 is generally acceptable for biodegradation; a pH of 6.5 to 8.5 is generally considered to be appropriate for optimal biodegradation efficiency. Soil pH also affects the availability of nutrients. The solubility of phosphorous, an important nutrient in biological systems, is maximized at a pH value of 6.5.

- Nutrients
  Microorganisms need nutrients, in particular nitrogen and phosphorus, to work optimally. The rate between carbon, nitrogen, phosphorus (C:N:P) is used to decide whether the availability of nutrients is sufficient. C:N:P should, according to Norris, et al. (1994), be between 100:20:0,5 and 100:5:1 for optimum conditions.

2.3.1 Sorption processes

Petroleum hydrocarbons (PHC) dissolved in ground water encounter different processes through which they can be removed from ground water. They can undergo sorption into mineral grains of soil and sorption by organic carbon in the soil, react in chemical precipitation, and be biodegraded by microorganisms in soil and by plants. Due to sorption, PHC are retained much more than the water that transports them through the aquifer. This effect is called retardation. Chemical and biological reactions decrease the concentration of PHC in the plume, but do not necessarily change the plume movement. This one is driven by advection and dispersion.

The concentration of PHC dissolved in water is determined by the one-dimension advection-dispersion equation:

$$\frac{\partial C}{\partial t} = \underset{\textit{dispersion}}{D\frac{\partial^2 C}{\partial x^2}} - \underset{\textit{advection}}{v\frac{\partial C}{\partial x}} - \underset{\textit{sorption}}{\omega\frac{\partial C^*}{\partial t}} + \underset{\textit{reaction}}{\left(\frac{\partial C}{\partial t}\right)_{rxn}}$$

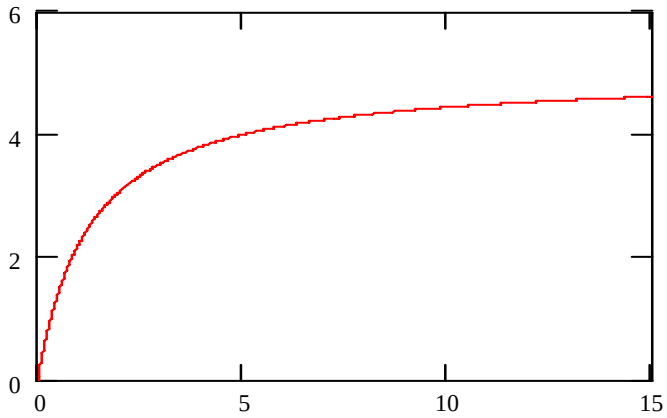
where: C = concentration of PHC in water
C^* = concentration of PHC sorbed in soil
D = longitudinal dispersion
v = average linear ground water velocity

The above equation is an example of the current understanding of the fate and transport of PHC in subsurface. Sorption can be subdivided to understand better the different processes that occur in the soil. Regarding PHC , it includes adsorption, chemisorption, and absorption. Adsorption is the phenomenon through which PHC stick to the grains of the soil. Chemisorption occurs when PHC are incorporated on a sediment, or a soil by a chemical reaction. Absorption occurs if soil particles are porous, PHC can then diffuse into the grains and be sorbed onto interior surfaces.

When these processes that define sorption are fast compared to the flow rate of water flowing through the soil, equilibrium is reached. The contaminant is partitioned between the water and the soil. This means that for a given concentration of PHC dissolved in water, the amount sorbed into the soil is defined. An equilibrium sorption curve can be built to determine the sorption capacity of the soil regarding different loads of pollutants in water. However, if sorption is slow compared to the water flow rate, equilibrium is not reached for the partitioning of the solute between soil and water. A kinetic sorption model is needed to describe the process.

2.3.2 Equilibrium sorption curve

Different models have been defined to represent the sorption of a solute onto soil. The Langmuir sorption model considers that soil offers a limited number of sorptive sites, so that when they are filled the concentration of PHC reaches a maximum. The equation of the model is:



$$C^* = C^*_{max} \frac{KC}{1 + KC}$$

Figure 1. *Equilibrium sorption curve*

C^*_{max} represents the maximum amount of PHC that can be sorbed by the soil, and K is a constant related to the binding energy. In particular cases where the sorption isotherm is linear, the slope of the graph is the partitioning coefficient K_p. It represents the ratio between the concentration of PHC in soil over the concentration of PHC in water:

$$K_p = C^*/ C$$

2.3.3 K_{oc}

Petroleum hydrocarbons have a very low polarity, they are difficult to dissolve in water and when they are dissolved, they tend to be attracted to surfaces less polar than water. Through that hydrophobic effect they can be sorbed onto pure mineral surfaces, but the primary adsorptive surface is the organic matter in the soil. If the organic carbon fraction, f_{OC} constitutes at least 1 percent of the soil in weight, the partitioning of PHC is considered to be almost exclusively onto the soil organic carbon (Fetter, 1999).

$$K_p = f_{oc} . K_{oc}$$

K_{oc} is the partitioning coefficient of the contaminant with respect to the organic fraction. This parameter can provide a good means of evaluation of K_p, when the sorption fulfills certain criteria. Firstly, sorption must be mostly hydrophobic as compared with chemisorption, or absorption. Secondly, organic carbon in the soil is the primary surface of sorption. Thirdly, the sorption isotherm is linear. Under these conditions, K_{oc} is correlated with fixed parameters such as K_{ow}, or solubility.

2.3.4 Correlation between K_{oc} and K_{ow}

Chu and Chan (1991) developed a correlation between K_{oc} and K_{ow}:

$$\log K_{oc} = 0{,}4735 + 0{,}7273 \log K_{ow}$$

This correlation provides an approximation of K_{oc}. However, uncertainties remain due to the empirical form of the determination ($r^2 = 0{,}79$) and the fact that the correlation was developed for a broad number of aliphatics.

2.4 Biodegradation of PHC

The process of transforming PHC to a non-hazardous compound in a biological system is a result of biochemical reactions in which microorganisms convert the hazardous compound to energy, carbon dioxide and water. The primary components required in a biodegradation process are microorganisms that can produce target-specific enzymes to degrade PHC, an energy source (PHC), and an electron acceptor for the redox reaction.

2.4.1 Enzymes

To make it possible for the microorganisms to use PHC as a carbon and energy source, it must be split up in smaller parts with the help of enzymes. Enzymes are complex organic proteins generated by microorganisms, which increase the speed of the redox reaction. Without these enzymes, chemical degradation may take years to occur because the activation energy necessary to trigger the redox reaction is too great. The enzymes, which act as a catalyst, accelerate the redox reactions by lowering the required activation energy (Maldini, 1994).

2.4.2 Electron acceptors

Biodegradation is basically an electron transfer process. When PHC are degraded, electrons are released and taken up by electron acceptors that differ depending on whether it is aerobic or anaerobic conditions. The quantitatively most important electron acceptor is oxygen, which works under aerobic conditions.

Oxygen works as an electron acceptor according to the equation:

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

Nitrate, Sulphate and Fe^{III} are some of the electron acceptors working under anaerobic conditions

Nitrate works as an electron acceptor according to the equation:

$$2NO_3^- + 12H^+ + 10e^- \longrightarrow N_2 + 6H_2O$$

2.4.3 Biological degradation of alkanes

Below is shown how enzymes can catalyse degradation of an alkane (C(O) stands for keto group C= O). First the terminal methyl group of the alkane is oxidised as in the equation. The resulting alcohol is then oxidised to the corresponding aldehyde and further to the carboxyl acid.

$$CH_3\text{-}(CH_2)_n\text{-}CH_3 \xrightarrow{1} CH_3\text{-}(CH_2)_n\text{-}CH_2OH \xrightarrow{2} CH_3\text{-}(CH_2)_n\text{-}C(O)H \xrightarrow{3} CH_3\text{-}(CH_2)_n\text{-}C(O)OH$$

Step 1 in the equation can be catalysed by the enzyme alkane-monooxygenase, step 2 by fatty-acid dehydrogenase and step 3 by fatty-aldehyde dehydrogenase. A large number of different aerobic microorganisms are capable to use the formed carboxyl acid under ß-oxidation and with the help of coenzyme (CoA)(Frick et al., 1999):

$$CH_3\text{-}(CH_2)_n\text{-}C(O)OH \xrightarrow[\text{ß-oxidation}]{\text{CoA, } H_2O} CO_2 + H_2O$$

This breakdown is called terminal oxidation but there also exist sub-terminal oxidation resulting in different oxidised by-products that is not completely mineralised (Larsson et al., 2001).

2.5 Biodegradability of Petroleum Hydrocarbons

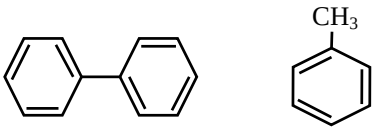
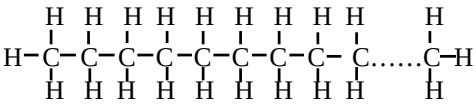
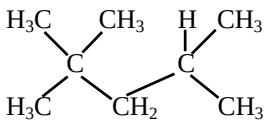
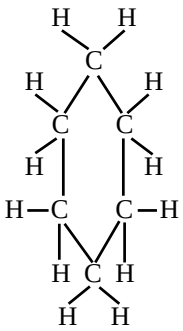
A very rough estimate of the relative biodegradability for some petroleum products, with the most easily degradable first, is:

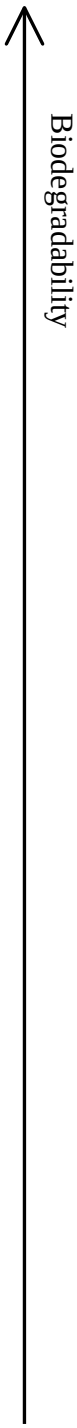
Gasoline ≥ Light heating oil ≥ Diesel > Lubricating oil > Heavy heating oil >> Refinery remainder >> Bitumen

Theoretically can all hydrocarbons be biodegraded but the time it takes to degrade the substances differs a lot depending on treatment, microbial breakdown potential, molecule structure and the compounds microbial availability. Since there are hundreds of different compounds in petroleum products with varying rate of biodegradation it can be difficult to predict the total rate of degradation for a petroleum product. Furthermore the levels of the different compounds in a petroleum product can vary between different manufacturers, which make a prediction of the degradation rate even more difficult. When estimating the rate of biodegradation for petroleum products, the chemical structure of the molecules in a compound should together with the microbial degradation activity form the base for prediction of biodegradation (Lindmark, et al., 1995).

Diesel is mainly composed of alkanes and aromatics, with n-alkanes in the interval C13-C20 being the major constituent. A compositional analysis of a low-sulphur grade diesel showed that 83.7 percent of the weight was alkanes and 16.3 percent was aromatics (Pereira et al., 2000). Beside the n-alkanes diesel also consists of cycloalkanes, branched alkanes, mono-aromatics and di-aromatics. In table 1 are different molecule groups, found in diesel, listed in terms of biodegradability (Lindmark, et al., 1995).

Table 1. *Different molecule groups, found in diesel, listed in terms of biodegradability.*

Hydrocarbons in the molecule area C_{10}-C_{22}, for aromatics, n-alkylaromatic and n-alkanes are in comparison easily biologically degradable. Hydrocarbons with the same form but with C_1-C_9 are also relatively easy to degrade but tend to be more toxic to the micro-organisms than C_{10}-C_{22}.	aromatic: n-alkylaromatic:  Biphenyl Toluene
Hydrocarbons in the molecule area C > 22, for aromatics, n-alkylaromatic and n-alkanes needs longer time to breakdown biologically than C < 22 with the same form. This is due to their extreme low solubility in water, where the breakdown takes place.	n-alkane: 
The more branched a hydrocarbon is the higher is the octane number and the more tertiary and quartary carbon atoms. These have a structure that tends to make biological breakdown more difficult.	branched hydrocarbon:  Isooctane
Cycloalcanes are harder to break down than corresponding n-alcane and aromatic. Cycloalcanes < C_{11} is very toxic to the cellmebrane of the micro-organisms.	cycloalkane:  Cyclohexane (C_6H_{12})



2.6 Phytoremediation

Phytoremediation is a relatively new technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. This technology has been receiving attention as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites (Schnoor, 1997). The major phytoremediation processes are: phytostabilisation, rhizodegradation, phytodegradation, phytovolatilization, phytoextraction, rhizofiltration and phytomining (US EPA, 2000). The experiments that were performed in this study focus on phytostabilisation and its possibility to reduce PHC in contaminated soil. The mechanisms for this method are described below and are followed by descriptions of other phytoremediation processes that can be used for reduction of PHC in soil and water.

2.6.1 Phytostabilisation

This mechanism is the use of certain plant species to immobilize contaminants in the soil, sediments, and groundwater through the absorption and accumulation into the roots, the adsorption onto the roots, or the precipitation or immobilization within the root zone. These chemical contaminants then are turned into in a stable form (US EPA, 2000). This is illustrated in figure 2 for an organic contaminant.

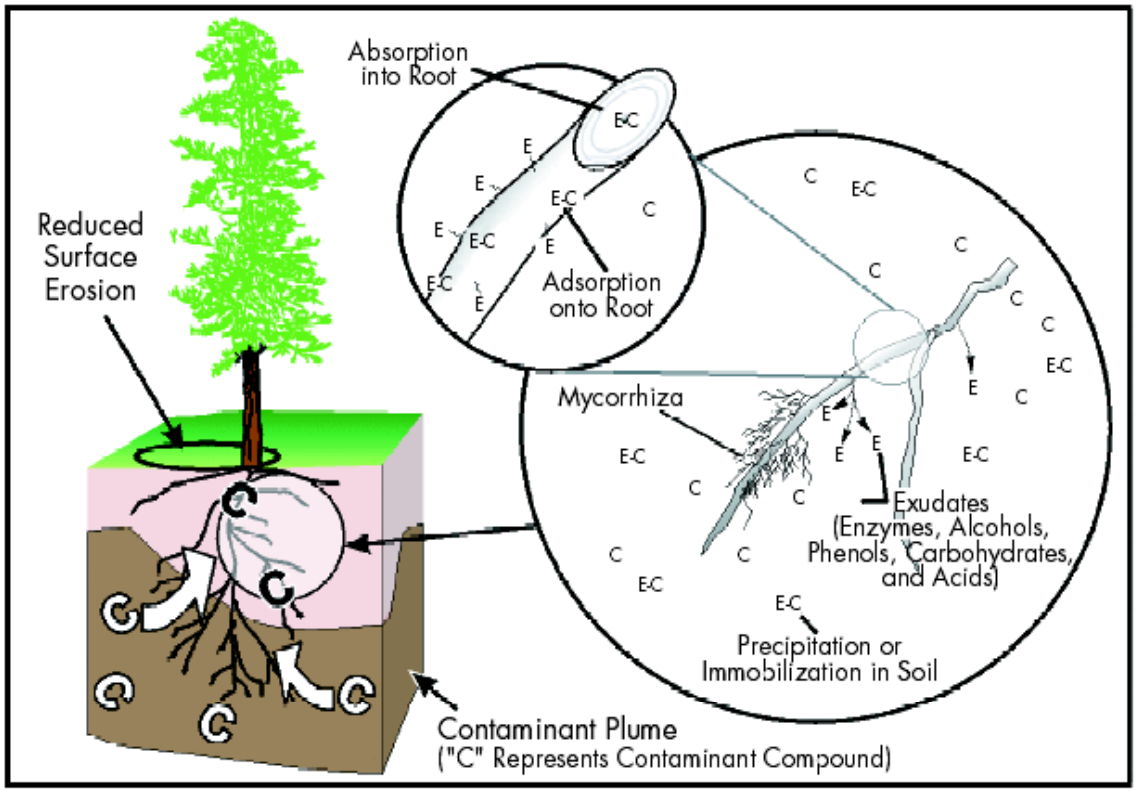


Figure 2. *Phytostabilisation of organics in soil (BNL Environmental Management Directorate, 2001)*

The three mechanisms within phytostabilisation that determine the fate of the contaminants are described in more detail below. These processes reduce the mobility of the contaminant and prevent migration to the soil, groundwater, or air.

- Phytostabilisation in the Root Zone:
  Proteins and enzymes produced by the plant can be exuded into the rhizosphere by the roots. These plant products target contaminants in the surrounding soil, leading to the precipitation or immobilization of the contaminants in the root zone. This mechanism within phytostabilisation may reduce the fraction of the contaminant in the soil that is bioavailable.

- Phytostabilisation on the Root Membranes:
  Proteins and enzymes directly associated with the root cell walls can bind and stabilize the contaminant on the exterior surfaces of the root membranes. This prevents the contaminant from entering into the plant itself.

- Phytostabilisation in the Root Cells:
  Proteins and enzymes also are present on the root cell walls that can facilitate the transport of contaminants across the root membranes. Upon uptake, these contaminants can be sequestered into the vacuole of the root cells, preventing further translocation to the shoots (Frick et al., 1999).

An indirect effect of phytostabilisation is the reduction of contaminant transport through erosion. Specifically, this technique can be used to stabilize contaminated sites by establishing a vegetative cover over areas where natural vegetation may be lacking due to high contaminant concentrations. Contaminant-tolerant species may be used to restore vegetation at the sites, thereby decreasing the potential migration of contamination through wind erosion, soil erosion, surface water runoff, and leaching of soil contamination to groundwater (Frick et al., 1999). In addition to using plants as vegetative covers, deep-rooted species, particularly trees, can be used to create hydraulic barriers to minimize or prevent groundwater and plume migration (US EPA, 2000).

2.6.2 Rhizodegradation

Rhizodegradation is the breakdown of contaminants in the soil through the bioactivity that exists in the rhizosphere. This bioactivity is derived from the proteins and enzymes that can be produced and exuded by plants or from soil organisms such as bacteria, yeast, and fungi. Organic contaminants as PHC can be directly metabolised by these proteins and enzymes, leading to the degradation, metabolism, or mineralisation of the contaminants. Furthermore, many of these contaminants can be broken down into harmless products or converted into a source of food and energy for the plants or soil organisms (Frick et al., 1999).

Alternatively, exudates released by the plant roots, like sugars, alcohols, carbohydrates, and acids, contain organic carbon that provides food for the soil organisms, which enhance their biological activities. These plant exudates stimulate the soil organisms to biodegrade the organic contaminants cometabolically. Another effect of the presence of plants is that it aids microbial biodegradation by loosening the soil and transporting oxygen and water into the rhizosphere (US EPA, 2000).

2.6.3 Phytodegradation

Phytodegradation refers to the uptake of organic contaminants from soil, sediments, and water with the following transformation by the plants. Depending on factors such as concentration and composition as well as the plant species and site conditions, an organic contaminant, like PHC, may be able to pass, to some extent, through the protective barrier of the rhizosphere. If this occurs the organic may then be biodegraded within the plant itself. In order for a plant to directly degrade a compound, it must be able to take that compound up through its roots. Plants transform organic contaminants through various internal, metabolic processes that help catalyse degradation. The contaminants are degraded in the plant with the breakdown products then stored in the vacuole or incorporated into the plant tissues (US EPA, 2000).

2.7 Salix

Salix viminalis is commonly used in Swedish short-rotation forestry as a crop for biomass production and as an alternative to grain production. In Sweden, short rotation forestry is practised as a means of replacing fossil fuels and reducing the anthropogenic emissions of carbon dioxide. The biomass is used for the heating of buildings. Willow clones have been selected for their high growth potential under Scandinavian climatic conditions with a production exceeding 10 t/ha per year (Rytter, 2001).

Salix viminalis is a large native shrub, growing up to 5 m high (figure 3). It has narrow leaves, at least 5 times as long as broad, which are dark green on the upper and pale green with short, silky hairs underneath. *Salix viminalis* blooms in April-May (Anderberg, 1999). It is adapted to disturbed environments with a high nutrient availability, where rapid resource acquisition and height growth are means of competition against neighbours and to escape browsing. Repeated disturbance, as severe browsing by mammals, has selected for below ground storage (mineral nutrients and carbohydrates) to support rapid regrowth of shoots (Bollmark, 2000).

In Sweden studies in phytoremediation has, so far, focused on the ability for *Salix viminalis* to accumulate metals, which has shown good results (Greger, 2000), and not so much on the reduction of organic compounds.

Figure 3. *Salix viminalis (Anderberg, 1999).*

3 Materials and methods

3.1 The continuous flow system

3.1.1 Materials

Salix viminalis was planted in cylinders with a diameter of 25 cm and a height of 110 cm (figure 4). The composition of the soil surrounding the plants was a mixture of sand, leca, natural fertiliser, bark, and peat soil. To avoid interference of algae production, caused by sunlight, the cylinders were clad with black plastic film. The soil mixture was 90 cm high and the artificial groundwater level was set 20 cm below soil surface.

All pipes used for input and output water was either PVC or silicon. Input water for both PHC-solution and nutrients was introduced from the top of the containers by glass rods, which was put 5 cm below soil surface and the output water was lead out from the cylinders through PVC pipes attached at the bottom.

Mixing of PHC and water was done in an open steel tank with the measure 100x55x50 (figure 4). To solve the diesel, 0.5 weight percent of methanol was added and a stirring rod was mixing the solution with 1360 r/min. A *Watson Warlow 302s* pump was used to distribute the PHC-solution to the containers and Glass rods was used in the steel tank for pumping up the water.

The nutrient used in the experiment was a standard flower fertiliser (*Blåkorn*). It was mixed with water in a cylinder shaped plastic tank with the diameter of 65 cm and the height of 45 cm, before it was introduced in the containers with pumps as described for the PHC-solution.

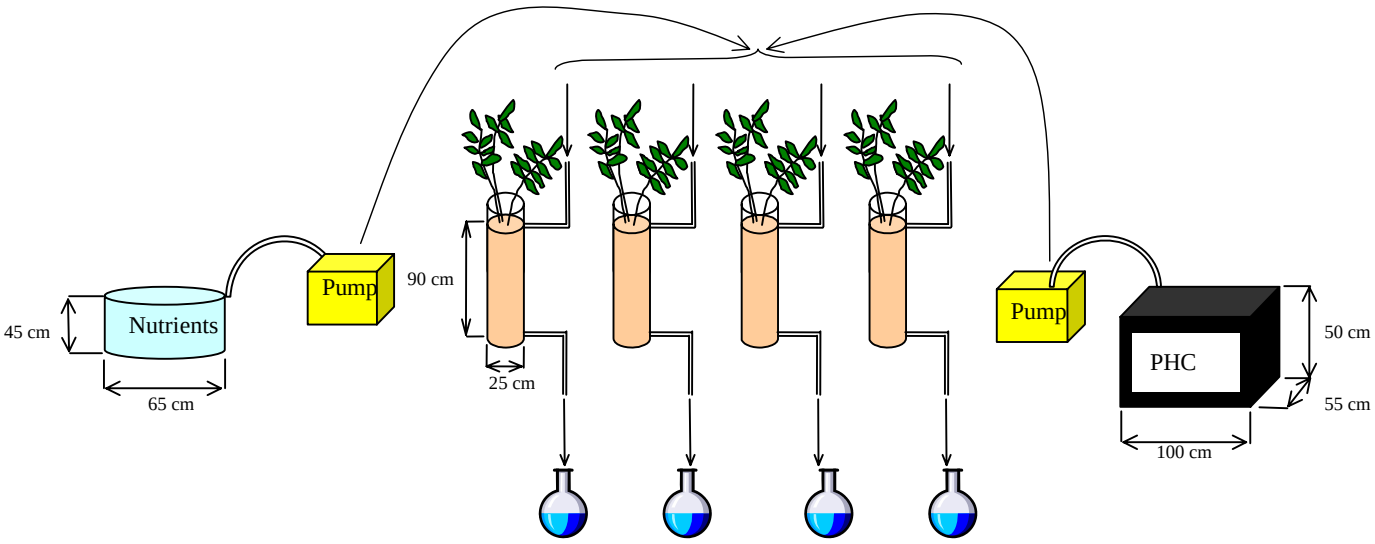


Figure 4. *System design of continuous flow system*

3.1.2 Flow rates and concentrations

In table 2 are presented the different flow rates and concentrations that were used in the experiments. The intention was to have two flow rates, high at 10 l/h and low at 3 l/h ; and three concentrations, high at 20 mg/l, medium at 10 mg/l and low at 2 mg/l. However, difficulties to set proper flow rates and problems when dissolving PHC in water caused some variations of the parameters.

Table 2. *Flow rates and concentrations*

Experiment	Flow [l/h]	Concentration in [mg/l]
High flow- High concentration	7	20
High flow- Low concentration	9	5
Low flow - High concentration	5	20
Low flow- Medium concentration	3.5	10
Low flow- Low concentration	2	2

3.2 Multiliner (pH, O_2)

To determine the oxygen content in the water a Multiliner P4 was used, which is an electrode instrument sensitive to dissolved oxygen. The electrode gives a signal that is proportional to the rate of diffusion of oxygen through a membrane, which is in turn proportional to the concentration of the oxygen in the sample (Reeves).

3.3 Infrared spectrometry

To measure the amount of PHC, infrared spectrometry was used. The principle of the method is that an infrared light beam passes through a cell (filled with the liquid to be measured) onto a radiation detector. The light intensity depends on the absorption that takes place when the beam passes through the cell. Infrared radiation is absorbed and converted by an organic molecule into energy of molecular vibration. This absorption is quantified, and a vibration spectrum appears as bands (figure 5). The spectrum of the sample is compared to a database of spectra of known compounds, which enables identification of the compounds in the cell. The frequency or wavelength of absorption depends on the relative masses of the atoms, the force constants of the bonds, and the geometry of the atoms (Silverstein et al., 1980).

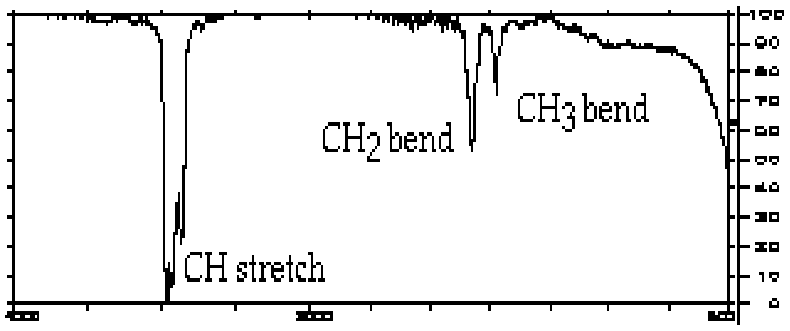


Figure 5. *Graphical display of Infrared Spectrometry (Aken,2002)*

The samples were kept in 1-litre glass bottles that were filled with 500-800 ml of the sample. To prevent microbial degradation of PHC in the samples, 3 ml of 8 mol HCl were added. The hydrocarbons were extracted from the acidified water adding a non-hydrocarbon solvent, TTE (1,1,2-triklor-1,2,2-triflouretan), to the samples that thereafter where shaken for 30 minutes in a shaking machine, *Stuart Scientific OKL 237*.

Infrared spectrometry was done according to the Swedish Standards Institution SS 028145 for determination of oil and similar compounds in water. This method determines the concentration of total extractable aliphatic compounds, total extractable aromatic compounds, unpolar aliphatic hydrocarbons and unpolar aromatic hydrocarbons. The detection limit for total extractable aliphatic compounds and unpolar aliphatic hydrocarbons was 0.1 mg/l and 0.2 mg/l for extractable aromatic compounds and unpolar aromatic hydrocarbons. The spectrometer used was a *Perkin-Elmer Spectrum One FT-IR* that was set to measure wavelengths between 2750 cm^{-1} and 3170 cm^{-1}.

3.4 Mass loss of ignition method

The soil sample was evaporated in a weighed dish and dried to constant weight in an oven at 105 °C for 24 h. After the evaporation the sample and the dish was weighed and placed in an electric furnace at 500 C^{o} for 6 hours. The sample was cooled in desiccators and weighed again. Determination of total solids and mass loss on ignition was calculated according to Swedish Standards Institution SS 028113 and the content of organic carbon was approximated as 58 % of the mass loss on ignition (the Bemmelen factor)(table 3).

Table 3. *Results from mass loss on ignition method*

	Total solids (%)	Loss on ignition (%)	Organic carbon (%)
Container 1, less vegetated	84.8	3.4	2.0
Container 3, vegetated	83.9	4.8	2.8
Container 4, less vegetated	88.0	2.6	1.5

4 Results and Discussion

The experimental design with a continuous flow system with downward flow showed to be appropriate for studying the containment of petroleum hydrocarbons in the soil. The system required relative low maintenance and showed to work properly for low and intermediate loads of contaminant. However in a longer term the permeability of the soil was seen to decrease, changing the flow of water in the soil.

4.1 Inflow solution

Diesel has low water solubility due to its composition with many unpolar hydrocarbons. According to the high proportion of longer n-alkanes ranging from C_{12}-C_{16} present in diesel, hexadecane ($C_{16}H_{34}$) was used to estimate the water solubility. This estimation is 10^{-4} mg/L (Pereira et al., 2000), which is under the detection limits for IR spectrometry used in the study. Furthermore that low amount of PHC in the input solution would not have been suitable for the aim of the study.

The solubility of diesel solutions can be increased by other organic compounds more hydrophilic that act as solvents. Methanol is a suitable cosolvent as it is an organic compound with a good hydrophility (K_{ow} = 0,2). Adding 0.3% methanol in weight, increased PHC solubility to suitable levels, about 60 mg/L. However this cosolvation changes the chemical properties of the inflow solution, it also alters the sorption and degradation of the contaminants.

4.2 Concentration and flow rate

The output concentrations were between 2 and 10 % of the input concentrations for all the experiments regardless of flow rates and input concentrations. The saturation capacity of the soil was not reached in any of the different experiments.

The trend of the output concentration over time was the same for seven out of nine experiments, with equilibrium reached between the amount sorbed and the water flowing out.

Comparing input and output levels for the cases with same flow rate but with one case having a high (20 mg/l) concentration and the other a low (5mg/l) concentration, the absolute levels are higher for the high input (figure 6). However the relative containment is better in the experiment with high input concentration (figure 7).

It is most likely that the difference of PHC in inflow compared to outflow is the result of different types of sorption or stabilisation. That this interference is due to rhizodegradation or phytodegradation is most improbable, since these processes in studies has shown a much slower process of PHC degradation with measurable dissipation in soil first after 2 to 3 months (Fiorenza, et al., 2000).

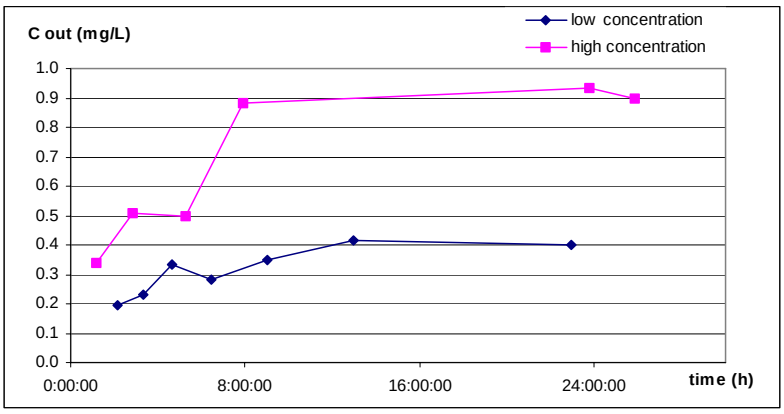


Figure 6. *Comparison of C_{out} over time between high flow-high conc. & high flow-low conc.*

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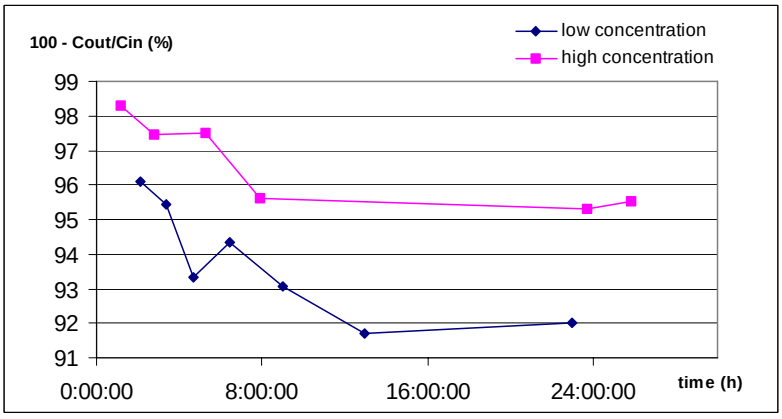

Figure 7. *Comparison of containment of PHC in the soil between high flow-high conc. & high flow-low conc.*

4.3 Sorption

A mass balance equation was used to calculate the mass of PHC accumulated inside the container (m*). 24 hours before each experiment the system was set under the appropriate water flow rate conditions to reach a continuous flow state for the test. Therefore no sorption of water occurred during the tests, so that the volume (V) of the container and the flow of water were considered to be constant ($Q_{in} = Q_{out} = Q$).

$$m^*(t) = \frac{Q}{V}\left(C_{in} \times t - \int_0^t C_{out}\, dt\right)$$

The integral of the concentration flowing out of the container was calculated by a trapeze method.

For those experiments where equilibrium was reached, a sorption curve could be established to characterise the sorption of hydrocarbons into the soil (Figure 8). The representation was linear with a good regression coefficient ($r^2 = 0{,}93$). From the slope of the regression, the partitioning coefficient was found to be $K_p = 36$.

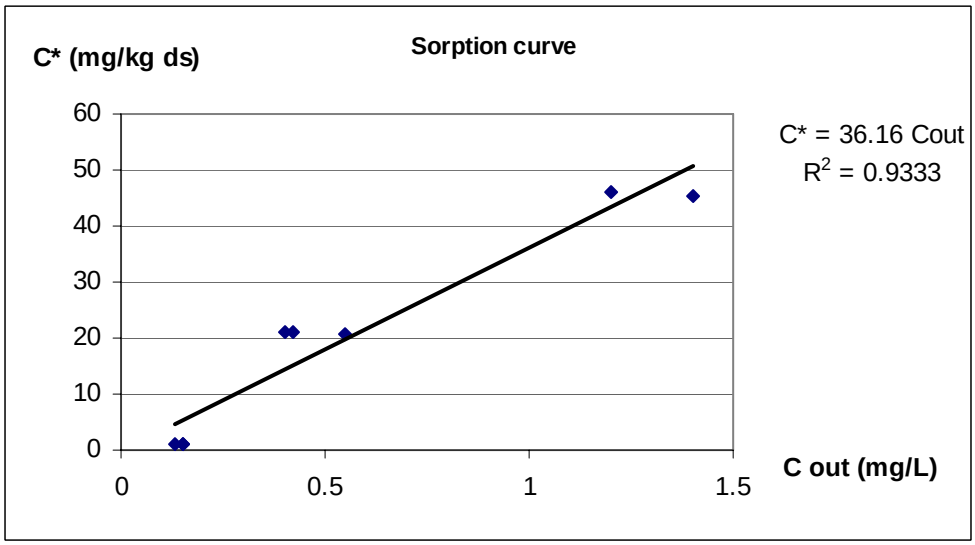

Figure 8. *Sorption Curve for Experiments*

In practical terms, this means that the affinity of the PHC for the soil phase is 36 times bigger than for the water phase.

To compare this result, K_p was calculated as described in chapter 2.3. The K_{ow} for Hexadecane was used to estimate K_{ow} for diesel:

$$\log K_{ow} = 8.25$$

$$\log K_{oc} = 0.4735 + 0.7273 \log K_{ow} = 6.5$$

The percentage of organic carbon was 2 % of the total dry weight of the soil.

$K_p = f_{oc} \,.\, K_{oc} = 6 \cdot 10^4$

This value is much higher than the K_p obtained from the experiment. The estimated K_p represents the partitioning between soil and water phases for hydrocarbons dissolved in only water. In the experiment, methanol was added to improve the solubility of hydrocarbons in water. Besides improving the solubility, it competes with the hydrocarbons for sorptive sites in the soil. This will decrease the partitioning coefficient for hydrocarbons in the soil.

Taking into account the effect of methanol for the partitioning between soil and water, K_p was recalculated. The fraction of methanol in the inflow solution was 0.3 % and the K_{ow}(methanol) = 0.2

$$\log K_{ow} = 2.5$$

$$\log K_{oc} = 0.4735 + 0.7273 \log K_{ow} = 2.3$$

$$K_p = f_{oc} \cdot K_{oc} = 4$$

Palmer and Johnson (1991) have described this effect with anthracene, whose partitioning coefficient decreased drastically as the fraction of methanol increased. This effect can be disadvantageous at contaminated sites where the lower partitioning coefficient could result in higher transport rates towards the ground water.

From the comparison of experimental K_p with literature K_p, a good accordance was seen within the range of experimental uncertainties. In addition the sorption curve was found to be linear and the sorption of petroleum hydrocarbons was mainly hydrophobic, on the organic carbon. The amount sorbed was decreased by the addition of methanol in the inflow solution.

4.4 Petroleum Hydrocarbon in the soil

In order to see the containment and the composition of PHC in soil and water, samples were sent to a commercial laboratory for GC-MS analysis when the experiments were ended. Comparing the composition of PHC in soil and inflow solution, the proportion of longer aliphatic chain was greater in the soil than in the water (table 3). This is due to their higher hydrophobicity, which enhances the attraction to soil. On the other hand the shorter chain are more soluble in water.

Table 4. *Result from GC-MS Analyses of soil and water samples*

Compound	Container no. 1				Container no. 3			
	Soil		Water		Soil		Water	
	[mg/kg ds]	[%]	[mg/l]	[%]	[mg/kg ds]	[%]	[mg/l]	[%]
Total TEX	< 0.1		< 0.001		< 0.1		< 0.001	
Aliphatic C5-C8	< 5		< 0.02		< 5		< 0.02	
Aliphatic >C8-C10	< 5		0.16	0.9	< 5		0.020	0.1
Aliphatic >C10-C12	64	11	4.0	23.3	88	8.5	1.90	12.2
Aliphatic >C12-C16	520	89	13.0	75.8	930	91.5	13.70	87.7
Total Aliphatics	584	100	17.16	100	1018	100	15.62	100

Both of the containers had high levels of PHC in the soil, especially longer aliphatic chains (C_{12}-C_{16}). When comparing the levels of PHC in the soil in the two containers, it was almost twice as high in container 3 (1018 mg/kg ds) as in container 1 (584 mg/kg ds). Since organic carbon in the soil is the primary sorptive compartment, the difference in PHC concentrations is likely due to the lower content of organic carbon in container 1 (2.0 %) than in container 3 (2.8%). The plants in container 1 suffered from parasite mites *(Tetranychus urticae)*, which led to their death, whereas the plants in container 3 lived throughout the experiments. The presence of plants could have affected the production of organic carbon in the soil, leading to increased containment in the soil.

Other studies have indicated that plants can enhance PHC containment when they have established sufficient root and shoot mass (Frick et al., 1999). Another aspect of phytostabilisation is the adsorption of PHC onto roots. This effect depends on the degree of hydrophobicity of the compounds. Highly hydrophobic chemicals (log $K_{ow} > 3.0$), like longer aliphatic chains, are strongly bound to the root surface due to the high proportions of lipids present there. Further transport beyond the surface is prevented by this strong affinity (Frick et al., 1999). Moderately hydrophobic chemicals (log $K_{ow} = 0.5$-3.0), like short chain aliphatics, can be transported from soil into stem via xylem. Trapp et al. (2001) studied this effect on *Salix viminalis* and found that intermediate hydrophobic chemicals are retained in the wood for a long time. Since the composition of the inflow solution was dominated by longer aliphatic hydrocarbons (table 3), its more likely that the PHC was sorbed to the root surface rather than taken up inside the plant. This decreases the possibilities for phytodegradation to occur.

Factors that affect biodegradation were monitored during the experiment. pH was near neutral values (6.9-7.3), which favours biological degradation (Norris, et al. 1994). The solubility of oxygen in water is often the limiting factor for aerobic biodegradation. The optimal level of oxygen content in water for biodegradation is around 10 mg/l (Larson et al., 2001). In this study, the oxygen content in water was between 3.4 and 6.9 mg/l and mostly around 4, which could have been a limiting factor for biodegradation.

Biodegradation could not be stated from the results of the study, although other studies have shown that degradation of PHC in soil is enhanced if plants are present. Fiorenza et al. (2000) treated an aged petroleum-contaminated soil with phytoremediation and the result was that degradation of PHC was greater in the vegetated areas compared to the unvegetated areas. In addition a plant growth chamber study indicated that PHC was degraded in the rhizosphere and degradation by-products ultimately became incorporated into the soil matrix.

In this study, the only phytoremediation process that could be seen was the enhanced stabilisation of petroleum hydrocarbons in the vegetated soil compared to the nonvegetated soil. Neither phytodegradation nor rhizodegradation was observed. Other studies have indicated that these two processes cold occur under certain conditions (Fiorenza, et al., 2000), (Frick et al., 1999).

4.5 Assessment of Phytoremediation on PHC-contaminated soil

Phytoremediation has proven to be a good method for cleaning up soils that have low or intermediate contamination of petroleum hydrocarbons. It is cheap in comparison with many in situ methods, like landfilling, thermal treatment and extraction/injection, and ex situ methods, like excavation (US EPA, 2000). Another benefit of phytoremediation is that the plants help contain the region of contamination by removing water from soil, thereby keeping the contaminants from spreading (Glass, 1999). An indirect benefit of phytoremediation is the improvement of soil quality by improving soil structure and increasing porosity, leading to better water infiltration, providing nutrients, and increasing soil organic carbon. Another indirect effect is the stabilisation and cover of the soil that prevents erosion and direct human exposure (Schnoor, 1997).

Although phytoremediation has shown good results in the remediation of petroleum-hydrocarbon-contaminated soils, there are limitations to the technology. Considerable time is needed to achieve regulated levels, depending upon the initial concentrations and the desired end point (Frick et al., 1999). Another limitation is the dependence on environmental conditions. Soil texture, pH, salinity, oxygen availability, temperature and level of non-hydrocarbon contaminants (e.g., metals) must all be within the limits tolerated by plants. In addition, phytoremediation of PHC may not work when concentrations of the contaminant is too high. The phytotoxicity of the contaminant can then prevent the plants to grow and thereby stopping the effects of phytoremediation (Fiorenza, et al., 2000). Another limitation is that PHC contamination must occur at shallow depths for phytoremediation to be effective. This is due to that the density of the root system decreases with depth leading to greater distance to contaminant. Furthermore if contaminants are tightly bound to soil particles or organic matter, they may not be available to plants or microorganisms for degradation (US EPA, 2000).

Phytoremediation of petroleum hydrocarbons in soil is an appropriate solution where the contaminant is present in the surface layer and the concentration is below phytotoxic levels for the plant. Furthermore, due to the time required for phytoremediation processes it is a suitable technology when the contaminant does not present an immediate danger to human health or the environment (Frick et al., 1999). The minimal amount of site management and low cost makes is particularly well suited to the treatment of large areas of contamination, when other methods may not be cost effective. Possible areas where it could be used is remediation of brownfields with intermediate surface contamination or as final polishing step in soil remediation.

5 Recommendations

For future studies on phytoremediation of PHC-contaminated soil with S*alix viminalis*, there are many interesting questions to answer. The following remarks could be taken into account to further develop the experiments performed in this study.

The influence of flow rates and concentrations on the sorption of petroleum hydrocarbons could be developed through higher concentrations and longer time span of the experiments. It would be interesting to reach the saturation capacity of the soil in order to know what loads of PHC *Salix viminalis* can tolerate. However, the problems encountered in this study highlight the fact that petroleum hydrocarbons are not easily dissolved in water, which makes it difficult to prepare higher loads of contaminants. Another limit is the permeability of the soil that is considerably reduced when PHC are sorbed into it. These compounds can form spots or ganglia of saturation that constitute a barrier for the migration of further pollution (Hunt,1988). This effect could be problematic when trying to reach the saturation.

After saturation is reached, a longer exposure (2-3 months) of soils to PHC-solutions could be developed to study the degradation of the compounds. Highly hydrophobic compounds have a strong affinity to the roots' surface that prevents them from an uptake into the plants. Therefore, phytodegradation is not thought to occur for this kind of compounds. Rhizodegradation is a more promising mechanism and could be further investigated, measuring the microbial activity and the appropriate conditions for microorganisms to degrade PHC.

6 Conclusions

Stabilisation of petroleum hydrocarbons in soil was shown to be increased with the presence of *Salix viminalis* compared to less vegetated soil. The plant increased the content of organic carbon in the soil. Since the sorption was found to be mostly hydrophobic on the organic carbon, the plants increased the number of sorptive sites for petroleum hydrocarbons present in the soil.

The effect of methanol in the experiment, exemplifies that compounds mixed with PHC can affect the containment of petroleum hydrocarbons in the soil. If these compounds are hydrophilic, like methanol, the affinity of PHC to soil would decrease and thereby the solubility of PHC in groundwater would be higher.

From the beginning of the study the intention was to reach saturation of PHC in the soil and thereafter study the possible phytodegradation and rhizodegradation. However problems with dissolving PHC in water limited the possibilities to study these mechanisms. Therefore biological degradation could not be observed.

The effect of phytostabilisation, seen in this study, is promising for remediation of low and moderately PHC contaminated soils. Other studies confirm that when time is not a limiting factor the contamination phytoremediation is an appropriate and cost effective remediation method.

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Appendix A Result from GC-MS

Results from GC-MS analyses at commercial laboratory (Analycen)

Table A1 *Analyses of soil samples*

Compound	Container no. 1 (D1)	Container no. 3 (L1)	Unit
Dry Substance	77.1	86.2	[%]
Benzene	< 0.01	< 0.01	[Mg/kg dry substance]
Toluene	< 0.1	< 0.1	[Mg/kg ds]
Ethylbenzene	< 0.1	< 0.1	[Mg/kg ds]
M/P/O-Xylene	< 0.1	< 0.1	[Mg/kg ds]
Total TEX	< 0.1	< 0.1	[Mg/kg ds]
Aliphatic C5-C8	< 5	< 5	[Mg/kg ds]
Aliphatic > C8 - C10	< 5	< 5	[Mg/kg ds]
Aliphatic > C10 - C12	64	88	[Mg/kg ds]
Aliphatic > C12 - C16	520	930	[Mg/kg ds]
Type of Oil	Light gas oil		
TOC	1.8	0.8	[% ds]
Lost of Ignition	3.2	1.4	[% ds]

Table A2 *Analyses of water samples*

Compound	C1 in (D1)	C1 out (D1)	C3 in (L1)	C3 out (L1)	Unit
Benzene	< 0.001	< 0.001	< 0.001	< 0.001	[Mg/l]
Toluene	< 0.001	< 0.001	< 0.001	< 0.001	[Mg/l]
Ethylbenzene	< 0.001	< 0.001	< 0.001	< 0.001	[Mg/l]
M/P/O-Xylene	< 0.001	< 0.001	< 0.001	< 0.001	[Mg/l]
Total TEX	< 0.001	< 0.001	< 0.001	< 0.001	[Mg/l]
Aliphatic C5-C8	< 0.02	< 0.02	< 0.02	< 0.02	[Mg/l]
Aliphatic > C8 - C10	0.16	< 0.02	0.020	< 0.02	[Mg/l]
Aliphatic > C10 - C12	4.0	< 0.10	1.90	< 0.10	[Mg/l]
Aliphatic > C12 - C16	13.0	< 0.10	13.70	< 0.10	[Mg/l]
Type of Oil	Diesel	No indication	Diesel	No indication	

Appendix B Results from IR spectrometry and Multiliner

High flow (7 l/h), High Concentration (20mg/l)

Container 1, less vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	1:10:00	670				0.37
2	2:48:30	610	7.2	4.44	44.5	0.42
3	5:14:30	630				0.39
4	7:53:00	750	7.25	2.8	28.5	0.36
5	21:47:00	680				0.67
6	23:45:30	690	7.35	2.4	22.5	0.84

Container 3, vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	1:10:00	660				0.34
2	2:48:30	740	7.1	3.23	32.7	0.51
3	5:14:30	630				0.5
4	7:53:00	760	7.07	3.38	33.7	0.88
5	23:44:30	710				0.935
6	25:50:30	690	7.25	2.85	27	0.895

Container 4,less vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	1:10:00	660				0.74
2	2:48:30	620				0.43
3	5:15:00	690				0.56
4	7:53:00	620				0.42
5	21:47:00	710				0.49
6	23:45:30	700				0.6

High flow (9 l/h), Low Concentration (5mg/l)

Container 1, less vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:58:00	900				
2	2:07:30	770	7.2	6.37	62	0.29
3	3:19:00	640				
4	4:39:30	780				0.28
5	6:26:30	760	7.15	5.8	57	0.26
6	9:00:00	640				0.32
7	12:56:00	650				0.42
8	22:56:00	640	7.3	4.59	46.2	0.52

Container 3, vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:58:30	650				
2	2:08:00	650	7.25	6.26	60.5	0.20
3	3:20:30	720				0.23
4	4:40:00	640				0.33
5	6:27:30	700	7.15	4.17	41.5	0.28
6	9:01:30	650				0.35
7	12:57:30	680				0.41
8	22:57:30	660	7.25	3.18	32.5	0.40

Container 4, less vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:58:30	670				
2	2:08:30	640	7.3	6.89	67.2	
3	3:20:30	700				
4	4:40:30	630				
5	6:27:30	650	7.25	6.1	60.5	0.35
6	9:03:00	660				0.40
7	13:00:00	600				0.42
8	23:00:30	640	7.25	4.59	47.3	0.41

Low flow (5 l/h), High Concentration (20mg/l)

Container 1, less vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:57:15	680				0.38
2	2:14:00	680	7.05	4.35	47.1	0.7
3	3:24:30	760				0.79
4	4:59:00	710				0.65
5	7:50:00	770	7.2	3.43	36.8	0.68
6	9:20:00					1.45
7	24:59:00	730				1.4

Container 3, vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:57:00	700				0.36
2	2:14:00	690	7	4.24	46.2	0.83
3	3:24:30	780				0.92
4	4:59:00					0.51
5	7:50:00	750	7.15	3.89	41.8	0.41
6	9:20:00	650				0.6
7	24:59:00	780				1.37

Container 4, less vegetated

no	*time [hr;min;sec]*	*V(ml)*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:57:00	680				
2	2:14:00	710	7.1	4.97	53.9	0.31
3	3:24:30	800				0.19
4	4:59:00	680				0.24
5	7:52:00	720	7.3	3.76	41	0.57
6	9:20:00	670				0.43
7	24:59:00	700				0.40

Low flow (3.5 l/h), Medium Concentration (10mg/l)

Container 1, less vegetated

no	*time [hr;min;sec]*	*V [ml]*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:17:45	950	7.18	6.06	58.2	0.11
2						
3						
4						
5			7.24	6.09	58.5	
6	2:09:30	660				
7	2:54:00	880				
8	3:32:00	600				
9	4:50:00	860				
10	5:56:00	770	7.19	6.00	57.8	0.06
11	7:11:00	800				
12	8:12:30	880		6.06	58.4	0.15

Container 3, vegetated

no	*time [hr;min;sec]*	*V [ml]*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:17:30	830	7.04	5.48	53	0.11
2	0:33:30	700				0.16
3	0:48:15	850				0.13
4	1:03:30	750				0.13
5	1:41:30	815	7.10	5.36	51.7	0.07
6	2:09:30	650				0.10
7	2:54:00	900				0.07
8	3:32:30	650				1.58
9	4:50:00	830				0.09
10	5:56:00	700	7.11	5.82	56.6	0.11
11	7:11:00	850				0.05
12	8:12:30	900		5.31	51.0	0.07

Container 4, less vegetated

no	*time [hr;min;sec]*	*V [ml]*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	0:17:45	750	7.16	5.14	49.7	0.11
2	0:33:30					
3	0:48:15					
4	1:03:30					
5	1:41:30		7.11	4.37	42.5	
6	2:09:30					
7	2:54:00					
8	3:32:00					
9	4:50:00					
10	5:56:00		7.17	4.42	43.0	
11	7:38:00	900		4.13	39.7	0.16
12	8:12:30					

Low flow (2 l/h), Low Concentration (2mg/l)

Container 1, less vegetated

no	*time [hr;min;sec]*	*V [ml]*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	1:05:38	690				
2	2:14:40	780	6.9	4.59	46.7	
3	2:59:53	875				
4	4:20:45	780				
5	5:35:00	750	7.05	4.66	49	
6	6:38:10	710				
7	8:49:40	730				
8	10:21:45	800				0.08
9	23:08:00	870	7.1	4.86	50.8	0.13

Container 3, vegetated

no	*time [hr;min;sec]*	*V [ml]*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	1:07:00	720				
2	2:27:02	900	7	4.92	49.8	
3	3:01:50	700				
4	4:25:00	700				
5	5:38:00	640				
6	6:41:47	610	7.1	4.88	51.2	
7	8:52:35	560				
8	10:21:45	570				0.16
9	23:16:30	650	7.15	5.41	57	0.15

Container 4, less vegetated

no	*time [hr;min;sec]*	*V [ml]*	*pH*	*O2 [mg/l]*	*O2 [%]*	*TPH [mg/l]*
1	1:07:15	700				
2	2:14:40	670	6.8	4.97	50	
3	3:00:38	700				
4	4:24:00	710				
5	5:38:00	780				
6	6:40:20		6.95	4.16	43.4	
7	8:49:20	560				
8	10:21:45	630				0.14
9	23:12:37	620	6.95	3.3	35	0.16

Appendix C Mass balance equation

$$Q_{in} C_{in} + r\,dV = Q_{out} C_{out} + \frac{dm}{dt}$$

The hypothesis assumed is that there is no chemical or biological degradation of PHC in the container during the time of the experiment. Therefore, rdV = 0.
The flow rate of water was constant throughout the experiment, $Q_{in} = Q_{out} = Q$.

$$Q_{in} C_{in} - Q_{out} C_{out} = \frac{dm}{dt}$$

The inflow concentration is constant in time, whereas the outflow concentration varies with time as seen in appendix B.

$$m = Q\left(C_{in} t - \int_0^t C_{out}\, dt \right)$$

The integration of the latter is approximated with a trapeze method where n represents the number of experimental points.

$$\int_0^t C_{out}\, dt = \frac{1}{2} \sum_{i=1}^{n-1} \left(C_i - C_{i+1}\right)\left(t_{i+1} - t_i\right)$$