



Transworld Research Network
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences, 2011: 223-246 ISBN: 978-81-7895-528-5
Editor: Diego Muñoz-Torrero

10. Biological remediation approach involving soils and groundwaters polluted with chlorinated solvents in a Mediterranean context

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Abstract. Chlorinated solvents such as trichloroethene (TCE) and tetrachloroethene (PCE) form a class of dense non-aqueous phase liquid (DNAPL) compounds that cause a persistent environmental contamination that is difficult to investigate and challenging to remediate. The use of vegetation to enhance degradation by soil microorganisms of chlorinated solvents in soil-groundwater systems (phyto and rhizo-degradation) has been receiving attention since the 90' as an attractive low-cost alternative. Remediation strategies must be site-specific, with separate approaches often warranted for the DNAPL source zone and its associated aqueous phase plume. It has been suggested that possible mechanisms for the enhanced bioremediation of TCE in the rhizosphere are excretion of phenolic compounds in root exudates and plant mycorrhization.

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Introduction

Tetrachloroethene (PCE) and trichloroethene (TCE) are among the most common groundwater contaminants of industrial origin; their usefulness lead to their wide use in many industries: electronics manufacturing, solvent production, pesticide / herbicide manufacturing, dry cleaning, instrument manufacturing, solvent recycling, engine manufacturing, steel product manufacturing, chemical production, rocket engine / fuel manufacturing, aircraft cleaning / engine degreasing, but also in industrial processes such as: metal cleaning, metal machining, tool and die operations, vapor and liquid degreasing or paint stripping; storage and transfer of solvents are also widespread activities [1]. Although PCE and TCE have been produced and utilized widely since the beginning of the 20th century, their importance as soil and groundwater contaminants was not recognized until the 1980s.

PCE and TCE, as well as carbon tetrachloride (CT) and chloroform (CF) are among the chlorinated solvents that form the primary classes of dense non-aqueous phase liquid (DNAPL) compounds. DNAPLs show common characteristics: a) immiscibility with water, forming separate phases, b) low absolute solubilities: they may persist for decades before dissolving in groundwater, c) relatively high densities, they can therefore sink beneath the water table, polluting the full thickness of the aquifer, and drinking water supplies, d) low viscosities, that allow rapid subsurface migration strongly affected by fluid pressures, which dictate the path the liquid will take, and e) toxicity.

PCE causes irritation of the upper respiratory tract and eyes, kidney dysfunction, and, at lower concentrations, neurological effects, such as reversible mood and behavioral changes, impairment of coordination, dizziness, headache, sleepiness, and unconsciousness. Short-term exposure to TCE causes irritation of the nose and throat and central nervous system (CNS) depression, with symptoms such as drowsiness, dizziness, giddiness, headache, loss of coordination; high concentrations of TCE have caused numbness and facial pain, reduced eyesight, unconsciousness, irregular heartbeat and death. CT is listed as a suspect carcinogen, a laboratory animal carcinogen at relatively high doses; however, human liver cancer has been reported; it can be absorbed through intact skin; it causes CNS depression, damaging kidneys, liver, or lungs, and can cause anemia, rapid and irregular heartbeats; health effects appear to be greatly increased by alcohol consumption. CF is a suspect carcinogen; it causes CNS depression, rapid and irregular heartbeat, and liver and kidney damage.

The concentrations in groundwater at which chlorinated solvents are harmful to human health are extremely low. The drinking water limit for common chlorinated solvents is 5 µg/L, and the groundwater quality standard is 10 µg/L.

They cause a persistent environmental contamination, which, in most cases, started decades ago. Some chemical manufacturer material safety data sheets distributed from the 1940s until the early 1970s suggested that 'acceptable practice' for the disposal of waste chlorinated solvents and the residues of distillation was to spread them onto dry ground/soil to allow them to evaporate. These safety data sheets recognized the volatile nature of many chlorinated solvents, but they did not recognize their ability to infiltrate rapidly into the subsurface, causing soil and groundwater pollution. Behind it is the widespread and socially accepted use of shallow soil systems as a location to dispose of unwanted materials.

The DNAPL source zone is defined as the area which has been in contact with DNAPL, and can include: 1) residual DNAPL, 2) pooled (free-phase) DNAPL, 3) DNAPLs sorbed to soil, and 4) dissolved contaminants diffused through fine grained media. Pollution is commonly widespread because plumes of dissolved-phase contamination emanate from the DNAPL source zones, where dissolution of DNAPL continuously supplies solute to the plume, defined as the contaminated groundwater emanating from the source (see Fig. 1).

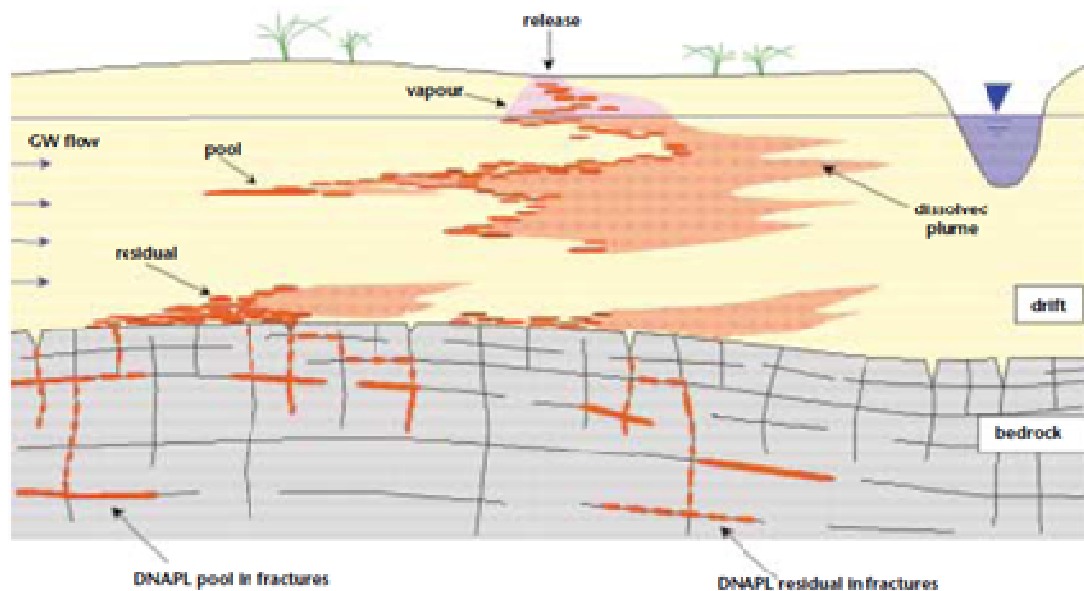


Figure 1. DNAPL source area, plume formation, and DNAPL accumulation in fractured media [2].

Natural attenuation that is naturally occurring physical, chemical, and biological processes, such as; biodegradation, dispersion, dilution, adsorption, and volatilization can be significant at some places. Plumes are retarded by sorption, especially in the presence of organic matter, but are also affected by abiotic and biotic degradation processes (see Fig. 2) and to a lesser extent by volatilization [3].

In aerobic environments, TCE and the metabolites of the reductive dehalogenation of PCE and TCE, such as dichloroethylene (DCE) and vinyl chloride (VC), are cometabolically oxidized to CO_2 by bacteria that possess oxygenase enzymes. Some of these enzymes are the methane monooxygenase (MMO) of methanotrophs, toluene dioxygenase (TDO) of *Pseudomonas putida* F1, toluene 2-monooxygenases (TMO) of *Burkholderia cepacia* G4, propane monooxygenase of *Mycobacterium vaccae* JOB5, phenol hydroxylase (PH) of *Alcaligenes eutrophus* JMP134 and *Burkholderia cepacia* G4, alkene monooxygenase (AMO) of *Alcaligenes denitrificans* spp., ammonia monooxygenase of *Nitrosomonas europaea*, and isopropylbenzene dioxygenase (IPB) of *Pseudomonas* sp. JR1 [4-11].

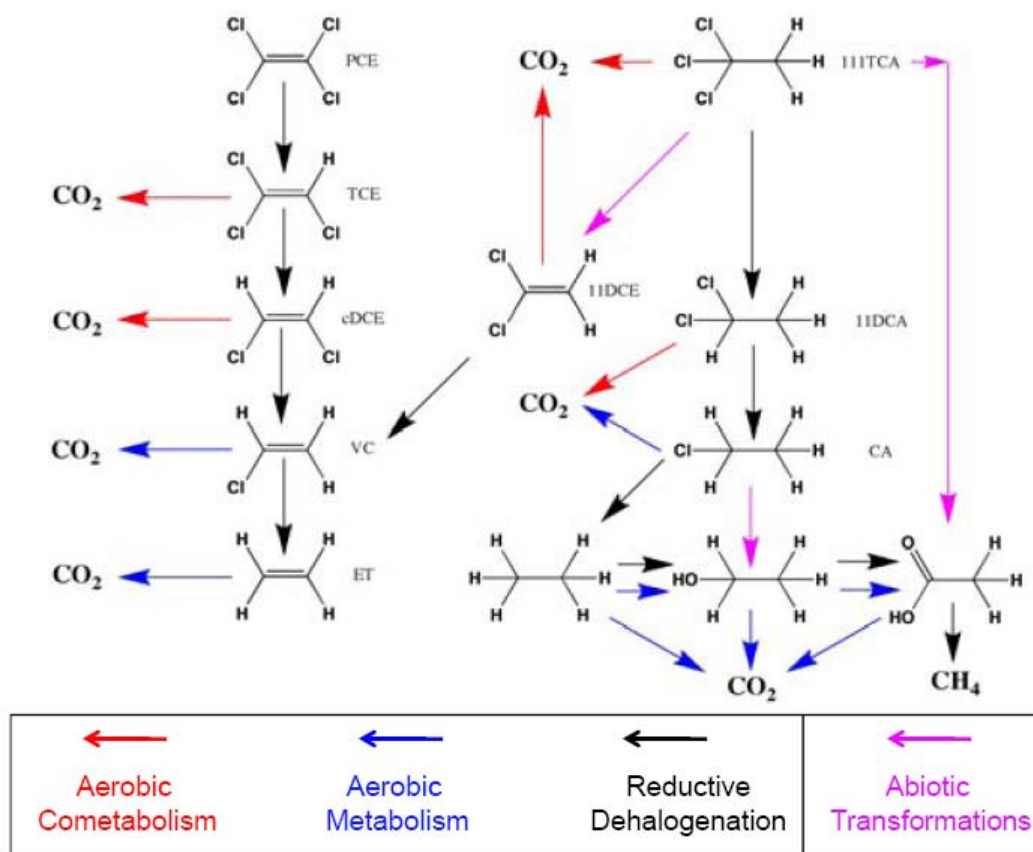


Figure 2. DNAPL main degradation processes.

TCE can be metabolized to vinyl chloride, a potent carcinogen, if oxygen is not present [12]; this degradation pathway is undesirable where VC can accumulate with no further breakdown.

Degradation of CT occurs slowly in the environment, indigenous microorganisms may degrade it to CF that may be more persistent than CT. CF is readily formed under anaerobic conditions [13]. Other degradation products, through reductive dechlorination, are methylene chloride (MC) and chloromethane (CM). For reductive dechlorination to occur, the water has to be anaerobic, i.e., dissolved oxygen must be less than 0.2 mg/L and redox potential should be less than -50 mV. Therefore, to deplete the oxygen present in the groundwater, organic substrates with sufficient or excess biochemical oxygen demand (BOD) have to be supplied. Further, electrons and hydrogen ions are necessary to replace the chlorine atoms. All these requirements are taken care of by organic compounds such as glucose, acetate, lactate, molasses, or vegetable oils.

Bouwer and McCarty demonstrated CT biodegradation in methanogenic and denitrifying environments with CF reported as a main metabolite [14,15]. Mayotte et al. identified a denitrifying organism, *Pseudomonas* sp. strain KC, that is capable of transforming CT without accumulation of significant quantities of CF [16].

Biodegradation is conditioned by the existence of a physical-chemical context and by a series of processes occurring in the soil and in the aquifer. For example, the presence of nitrates and/or sulphates could inhibit PCE biodegradation process, as has been noted by He et al. [17]. Moreover, textural characteristics and subsurface heterogeneities can also exert their influence on the biodegradation rates.

The use of vegetation to enhance degradation of chlorinated solvents in soil-groundwater systems (phyto and rhizo-degradation) has been receiving attention since the 90' as an attractive low-cost alternative to the traditional engineering approaches of soil excavation, incineration and air stripping. Conventional groundwater remediation methods, as "pump & treat", are not always cost-effective and can even provoke negative impacts such as: a) expansion of the source zone due to mobilization of residual DNAPL, b) undesirable changes in the DNAPL distribution, c) undesirable changes in physical, geochemical, and microbial conditions, d) adverse impact on subsequent remediation technologies, and e) increased life-cycle costs of site cleanup.

When plants are used, a series of mechanisms are involved, including phyto-volatilization, -accumulation, -degradation, -stabilization, and rhizodegradation. The role and contribution of each of these processes to the overall bioremediation system has not been accurately characterized [18,19].

Soils have many properties that can be dramatically modified at the rhizosphere, giving rise to the so called rhizosphere effect. Rhizosphere exhibits a unique biophysics and biogeochemistry which is different from that of the bulk soil. Rhizosphere responses to seasonal changes, including plant succession, rainfall, and temperature, can significantly influence potential PCE, TCE and CT bioremediation. Chlorinated solvents in soil must be in solution to be absorbed, modified, degraded, or sequestered by either plants or microorganisms.

Water movement and nutrient availability in the rhizosphere is a critical factor as plants take up many times more water than is needed for metabolism and growth. However, all of this water and compounds, dissolved in the soil solution, moves through the rhizosphere, where it is subjected to processing by microorganisms before it enters the roots.

Some rhizosphere microorganisms secrete plant hormones that increase root growth, and thereby the secretion of root exudates that contain metabolites, including proteins and carbohydrates, used by the bacteria. Exudates also contain natural chelating agents such as citric, acetic, and other organic acids that make not only ions of nutrients but contaminants more

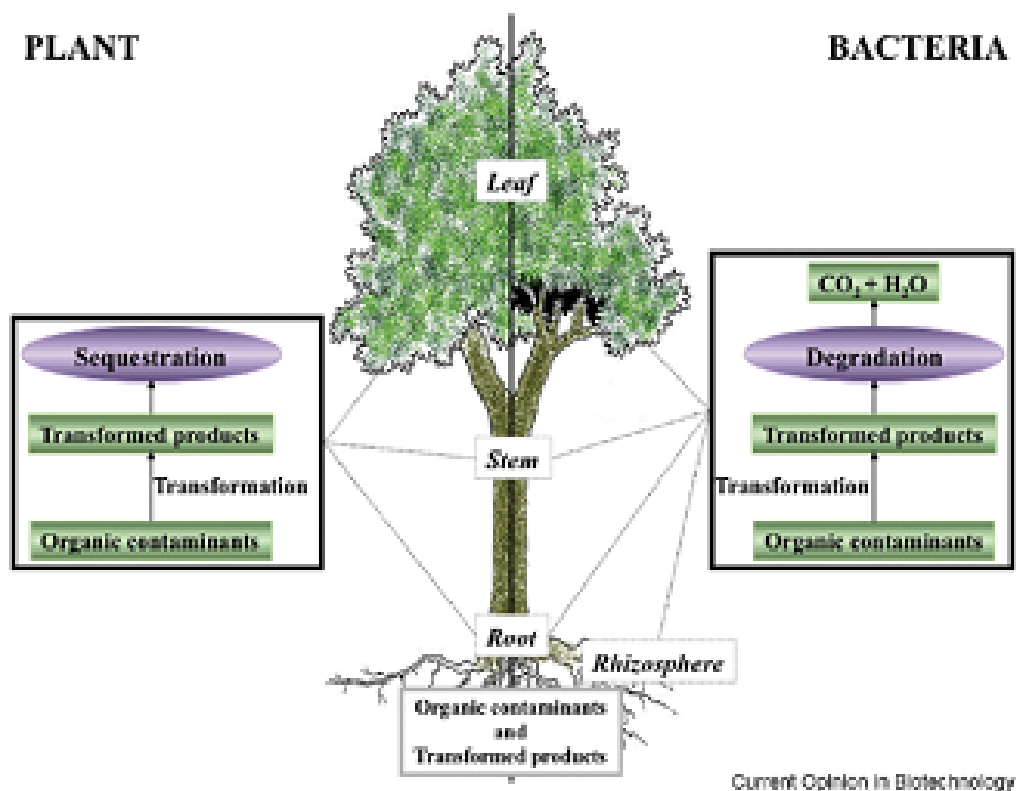


Figure 3. Contribution of plants and their associated bacteria to DNAPLs' phytoremediation.

mobile in the soil. Exudates may also include enzymes, such as nitroreductases, dehalogenases, and lactases. These enzymes have important natural functions, but may also degrade organic contaminants that contain nitro groups or halogenated compounds. It has been suggested that a possible mechanism for the enhanced microbial mineralization of TCE in the *Lespedeza cuneata* rhizosphere soil is excretion of phenolic compounds in root exudates. Since phenol is a known inducer of toluene monooxygenase, an enzyme responsible for degradation of TCE, the natural plant exudates could play a role in biodegradation of TCE in the rhizosphere [20]. Exudates have also been found to increase the TCE sorption capacity of soil [21].

The main uncertainty in phytoremediation of chlorinated solvents regards the magnitude of plant uptake, phyto-volatilization, and rhizodegradation. Several studies have reported that TCE disappearance in planted systems is mainly due to plant uptake, followed by phyto-volatilization and diffusion through the stem and/or metabolism by the plant [22]. On the contrary, other studies have observed TCE degradation occurring mainly as a result of rhizosphere microbial metabolism [18]. Uptake and dechlorination of CT by plant tissues is likely the primary mechanism for phytoremediation by poplar [23].

Phytoremediation of chlorinated solvents from soil and groundwater have reported up to 90% contaminant removal by the use of different plant species [24-26].

Poplar and willow trees are the preferred plant species in temperate climates for PCE, TCE and CT phytoremediation. Valuable poplar and willow common characteristics that make them ideal for this applications are that they are fast-growing, easily propagated, tolerant to relatively high levels of contaminants (<550 ppm TCE), resistant to saturated conditions, and they are phreatophytes, that is deep-rooted plants where water uptake is mainly from the groundwater [27]. Willows have been found to consistently utilize groundwater sources even during periods of rainfall [28]. Their transpiration rates can increase from 19 to 200-1000 L of water day⁻¹ in young to mature trees [27,29]. With these high transpiration rates they can extract enough water to depress the water table locally, inducing flow toward the trees and containing the contaminant plume (hydraulic control).

At recent pollution episodes it must be taken into account that TCE is acutely toxic to a variety of crop plants at concentrations of about 2 mM in the gas phase [30].

Box indicated that black willow and eastern poplar are capable of decontaminating solution concentrations of up to 20 mg/L of PCE and 45 mg/L TCE [31]. High concentrations of 45 mg/L PCE were shown to be toxic to the plants. Poplars are able, according to Gordon et al., to survive when grown in water containing 50 ppm TCE [32].

Poplar trees possess endophytic bacteria, including methanotrophs, that live symbiotically within the plants. Some of these bacteria that include members of *Pseudomonas sp.*, *Enterobacter-Clostridium* species, and methylotroph species such as *Methylobacterium populi sp. nov.* are known for their bioremediation potential [33-35].

The effectiveness of chlorinated solvent phytoremediation by poplar and willow trees is strongly influenced by the choice of genotypes (clones) that must be compatible with the intended use, site characteristics (soil type, microclimate, pests and diseases), and with the expert opinion concerning use of native versus exotic plants [36].

Other potential tree species that have been studied for chlorinated solvent phytoremediation are conifers [37]. Loblolly pine (*Pinus taeda*) is capable of up to 90% TCE removal from soils and groundwater at some areas [25]. This species is characterized by the production of significant quantities of oil extracts, composed mainly of monoterpenes. Therefore, these compounds may influence the microbial processes occurring in the rhizosphere, either as root exudates or leachates from the decaying foliage at the soil surface.

Schnabel *et al.* investigated in 1997 the uptake and transformation of TCE by edible garden plants: carrots, spinach, and tomatoes [21]. 74% to 95% of TCE was volatilized through the leaves and 5% to 25% was sorbed to the soil. 1% to 2% was found in the plant material and could not be extracted.

Schnoor proposed that TCE metabolism in plants is the result of dehalogenase degrading enzyme that oxidizes alkanes, alkenes, methanes, and their halogenated analogues [38]. An antibody assay has been developed to determine the presence of the dehalogenase enzyme in plants. This technique can be used to aid in the selection of viable plants for a particular site [39]. Cunningham *et al.* stated that many of the enzyme systems present in mammalian metabolism of TCE are also present in plants (e.g., cytochrome P-450 and glutathione) [40]. Schnabel *et al.* suggested that transformation of TCE after plant uptake may be similar to the mammalian breakdown of TCE. This theory is based on similar metabolites being observed in both plants and animals [21].

Soil and groundwater remediation are undertaken in European countries to manage unacceptable risks to human health and/or the environment. The application of more sustainable remediation technologies that do not require large amounts of inputs (inorganic fertilizers, surfactants,...) and promote the application of biological systems to solve anthropogenic environmental problems are highly recommended if knowledge about soil-plant system is as complete as possible.

Experience from the past years has demonstrated that DNAPL contaminated sites are difficult to investigate and challenging to remediate.

DNAPL can penetrate fractured rock and clay forming pools and, for this reason, many decades are required to dissipate DNAPL secondary sources in most hydrogeological environments. It is intended to assist site investigators, site owners and regulators in conducting site investigations and in selecting sustainable remediation approaches.

1. Mediterranean area and chlorinated solvent pollution

At least 4 attributes make the Mediterranean area indeed different and largely determine the nature of its soils: I) specific climate, II) topography and mountains, III) dust from the desert, and IV) long term effects of man.

The soils with such singular characteristics extend over 420 million ha approximately; they are old (product of interactions that have taken place for millennia between the natural processes of pedogenesis and the human activities), frequently polygenetic, affected by climatic fluctuations and man's management of landscape. Some soils are polycyclic, namely, composed of the superposition of several young soils, and resulting from the succession of several phases of pedogenesis over very long periods of time; others are, by contrast, truncated, amputated by the erosion of their surface horizons. Dry summer periods frequently coincide with the occurrence of violent rainstorms, causing average yearly soil losses above 15 tons/ha in more than one third of the Mediterranean basin. Such unreasonable soil losses drive a principle mechanism of irreversible soil degradation that disperses away pollutants when present.

Loss of organic matter and fertility, erosion, salinization, chemical pollution and its related problems have affected these soils since land was first settled and cultivated.

The annual rate of loss of organic matter varies greatly. High temperatures during Mediterranean summer lead to a rapid decline in the organic matter content, especially in cultivated soils.

Preliminary estimates of the UE indicate that 74% of the land in Southern Europe is covered by soils containing less than 2% organic carbon (less than 3.4 % organic matter) in the topsoil (0-30 cm) (see Fig. 4). Global climatic change (GCC) may further decrease organic matter content and soil biological activity.

Solvent production and metal degreasing are processes that are done in a great number of small plants. Concentrations of these plants in a certain industrial area may be considered as point sources at the regional level. In Catalonia, Spain, a representative industrial area of the Mediterranean basin, there are 20 main point sources of halogenated solvents.

A quarter of the inquiries opened in Catalonia because of groundwater pollution are due to chlorinated solvent releases (internal information of the Catalan Water Agency). Information about total volume of DNAPL released into soils and groundwaters during the last 30 years is not available.

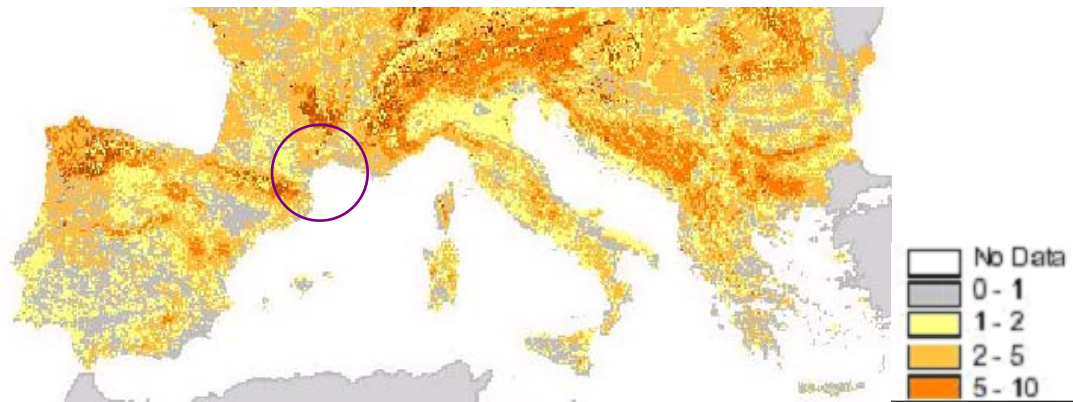


Figure 4. Organic Carbon Content (%) in topsoils of South Europe (UE, 2003).

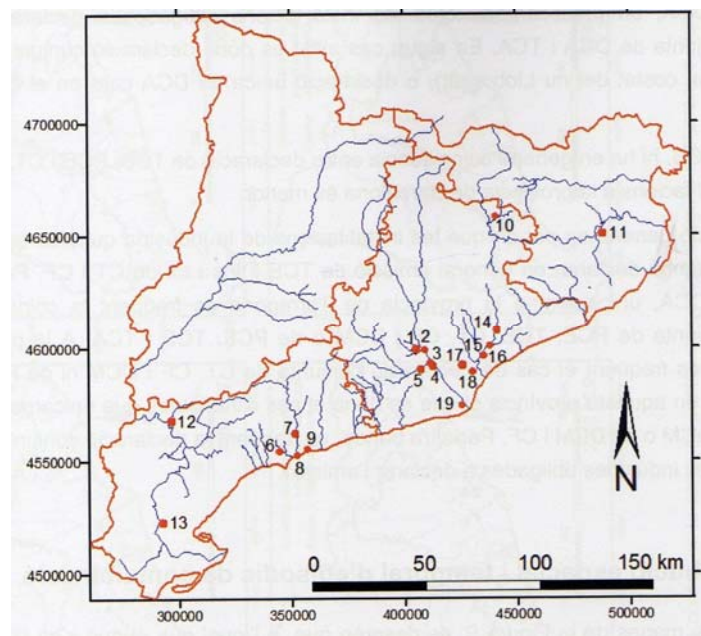


Figure 5. Chlorinated solvents production distribution at Mediterranean river basins in Catalonia [41].

2. Site and pollution characterization

Two polluted areas have been chosen to conduct site investigations and to study phytoremediation viability.

Site A

The site is located at a Quaternary alluvial irregular overburden varying in thickness between 3 and 12 m. The geological formation exhibits an important textural variability, consisting on fine sands and silts crossed by paleochannels made up of silty sands and gravels and sandy gravels with thin silty layers interbedded. These materials are distributed on terraces that are geomorphologically differentiated but hydraulically connected. These alluvial materials overlie a thick formation of Eocene fractured marls and marlstones containing some amounts of microscopic pyrite.

Natural soils show a stratified profile formed by recent deposits; organic matter decreases irregularly with depth and, on the average, is less than 1% in average. Soils are calcareous and mildly or moderately alkaline. They have good or moderately good drainage.

The upper layer consists of a horizon about 35 cm thick subjected to the influence of human activities, more or less nutrient enriched but structurally fairly good. Underneath this horizon, down to a variable depth, is to be found the rest of deposits, unaffected by ploughing, which may be considered as C horizon, and that can be penetrated by roots. Soil materials are derived from erosion of pre-existing soils, almost all calcareous in character.

Approximately 700 mm rainfall per year, with a total maximum daily of 125 mm in autumn, is collected on the area. During the dry period, rainfall does not cover evapotranspiration losses. The depth to the water table ranges from 2 to 8 m. The alluvial formation constitutes an unconfined aquifer connected to the Ges River.

Groundwater contamination was detected in 2000 at a municipal water supply well at levels of 70 µg/L of PCE. The polluted well was closed in January 2001 by the Catalan Hydraulic Administration (ACA), which ordered to do the first works of characterization and rehabilitation.

The groundwater contaminant plume was already displayed in 2001 showing variations from 1,700 to 10,300 µg/L of PCE, and between 38,000 and 338,000 µg/L of PCE, depending on the two source areas. A third area, at the right hand side of the river, was also individualized; this source was caused by a partial lateral migration of PCE free phase through a paleochannel connecting both banks of the river. Currently, the river cuts the Eocene marls but near the last sources it cuts the paleochannel.

The length of the plume was about 1,400 m, with ending concentrations that range between 50 and 200 µg/L of PCE plus TCE; the calculated affected water volume was about 200,000 m³. The applied methods of rehabilitation have been pumping and, air stripping in one case, active charcoal in another.

Since 2005 our team is carrying out studies to characterize adequately the episode rehabilitation fate, in order to improve rehabilitation strategies.

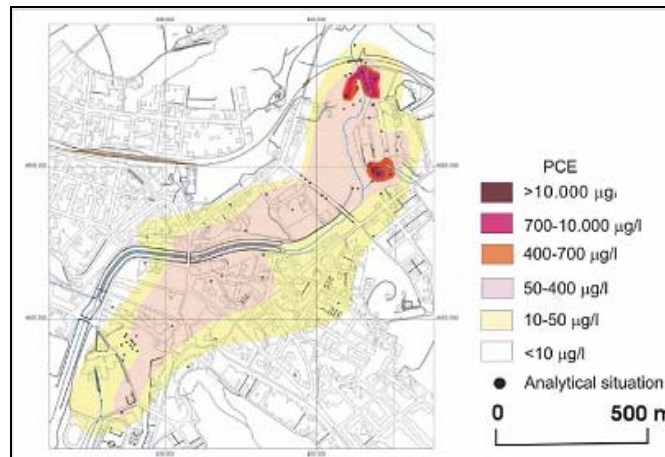


Figure 6. PCE plumes emerging from source areas in July 2001 (ACA).

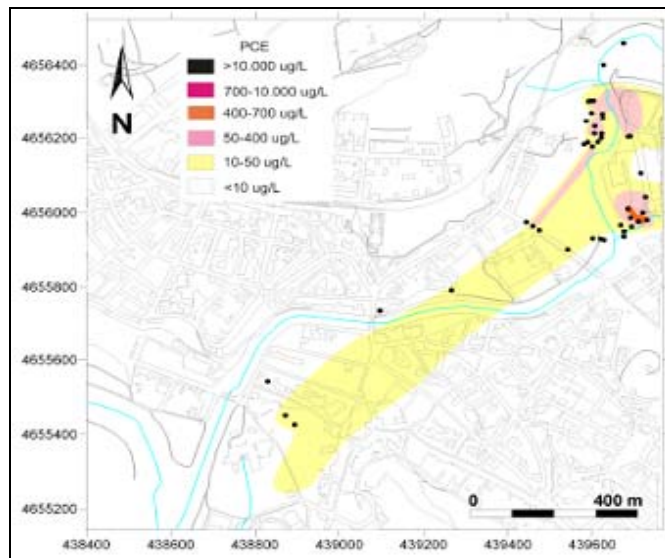


Figure 7. PCE plumes emerging from source areas in July 2006 (ACA).

Table 1. Groundwater physical-chemical parameters determined bimonthly during two years (2005-2007) in sources and downstream, in the proximity of the sources.

	Left bank (River Ges)								Right bank (River Ges)			
	Source area A				Source area B				Source area C			
	min	max	mean	v.c.	min	max	mean	v.c.	min	max	mean	v.c.
pH	6.85	8.01	7.24	0.04	6.87	8.14	7.43	0.04	6.8	9.34	7.58	0.05
Eh	-108	406	137.4	0.75	-27	197	112.6	0.70	-120	646	87.24	1.31
DO	0.2	5.4	3.06	0.58	1.5	10.8	4.17	0.67	0.22	4	1.2	0.87
T	10.2	18.5	14.51	0.1	11.7	19.8	15.42	0.13	2.5	20.9	13.5	0.34
CE	493	2930	964.6	0.5	436	3830	1191.	0.71	325	2780	638.1	0.7

Eh: redox potential, DO: dissolved oxygen, T: temperature, CE: electrical conductivity CE (µS/cm).

Table 2. Groundwater chemical parameters determined bimonthly during two years (2005-2007) in sources and downstream, in the proximity of the sources. Concentration is expressed in mg/L.

	Left bank (River Ges)								Right bank (River Ges)			
	Source A				Source B				Source C			
	min	max	mean	v.c.	min	max	mean	v.c.	min	max	mean	v.c.
HCO ₃ ⁻	266	734	358	0.2	234	755	343	0.4	140	1247	301	0.5
SO ₄ ²⁻	65.5	1027	180	1.0	37.5	661	220	0.9	5.5	2352	121	2.3
Cl ⁻	15.1	123	41.7	0.6	7.76	398	69.7	1.4	6.24	73.7	18.1	0.7
Na ⁺	1.3	450	53.5	1.7	13.1	259	98.4	0.9	7.7	1861	103	2.5
K ⁺	4.1	13.5	7.4	0.3	3.8	96.8	19.2	1.4	0.05	49.0	6.1	1.1
Ca ²⁺	84.8	277	148	0.3	69.1	511	153	0.7	15.9	390	83.3	0.6
Mg ²⁺	15.6	35.0	27.7	0.2	13.8	115	32.0	0.7	5.4	71.4	17.2	0.4
NO ₃ ⁻	7.7	525	93.1	1.0	ND	838	169	1.2	ND	3044	109	4.0
NO ₂ ⁻	ND	0.26	0.01	3.9	ND	0.6	0.04	3.4	ND	62.5	2.3	4.6
NH ₄ ⁺	ND	3.45	0.18	2.9	ND	18.3	2.02	2.4	ND	20.5	0.41	5.4
TOC	0.89	6.10	2.31	0.5	0.71	10.7	2.20	1.2	1.20	11.9	3.13	0.7

At the source areas, PCE was present at a range of 99% of the molar fraction. Chloroethenes concentrations (PCE, TCE) in the plume show considerable spatial and temporal variability. Concentrations increased during the water table recovery periods because of the dissolution of chloroethenes from pools or trails of residual phase in the water saturated zone. Chloroethenes from marlstones, where they remain pooled at the fractures, can contribute to increasing levels in a percentage that ranges from 1 to 10, depending on the punctual content at the two geological formations. The variability is also due to the influent character of the River Ges, which produces a dilution of contaminants. Concentrations of PCE, TCE and cDCE in the plume vary throughout the year, ranging from 0.3 µg/L of PCE, 0.11 of TCE and 0.1 of cDCE at periods in which rainfall infiltration and recharge from the river occur, to 950 µg/L of PCE, 410 of TCE and 940 of cDCE when only recharge from the river takes place.

The applied remediation system (pump and treat) led to an increase in flow velocity, which favored a greater DNAPL dissolution rate and consequently a greater bioavailability.

During our studies, degradation of PCE has been detected in some observation wells (piezometers). Vinyl chloride (VC), which is practically absent in the source areas, has been appearing at the plume in some points.

The particular spatial distribution of anaerobic microorganisms consortia detected during our tests, the prevalence of reducing conditions, the progressive decrease in PCE along the plume together with the increase in cDCE and VC as well as the progressively less negative $\delta^{13}\text{C}$ values [41] are signs of the

biodegradation of chloroethenes in reducing conditions (reductive dehalogenation).

A competition for electrons between chloroethenes and other electron acceptors with a greater reduction potential such as nitrates or sulphates has been detected.

A high nitrate background characterizes the regional hydrochemical context, because of the practice of widespreading pig manure in the catchment basin. The spreading usually takes place on December-January and October-November, as recommended by the Catalan Department of Agriculture; this fact explains the presence of denitrifying microorganisms. Because of electron competition between dehalogenating microorganisms and those responsible for denitrification, the rate of chloroethenes biodegradation assessed in the field was not high.

A continuous input of sulphates has also been observed. This input comes from oxidation of disseminated pyrites contained in the fractured marlstones.

Site B

The area is located at a small sedimentary basin, a tectonic depression filled with Plio-Quaternary detritic materials, originating from the dismantling of the adjacent reliefs. The thicknesses of this Quaternary formation do not exceed 14 m in the study zone, although in some boreholes drilled by the Spanish Geological and Mining Institute (IGME) values as high as 100 m have been recorded. Beds of heterometric gravels and sands with medium to coarse granulometry predominate; these are paleochannels of alluvial origin with a silt-clay matrix, alternating with silt and clay, together with some levels of caliche crust, which is a hardened calcium carbonate.

Natural soils are related with river sediments. The deposits were formed under flow conditions that were almost torrential; hence the predominance of gravels and rounded stones attaining considerable size sometimes. The soil is about 50 cm thick, or even less. The soil profile is differentiated from the geological materials by the passage into gravels. The presence of calcium carbonate is the general rule. The organic matter content is around 1%.

The area receives approximately 517 mm rainfall per year, with a total maximum daily of 113 mm in spring or autumn. During the dry period, rainfall does not cover evapotranspiration losses. The depth to the water table ranges from 7 to 8 m.

The alluvial fan deposits constitute a surficial aquifer formation, consisting of gravels and sands of different granulometry, structured in paleochannels, showing alternating silts and clays, not more than 14 m thick.

It is a free aquifer which is recharged throughout the area where its materials reach the surface; for this reason the aquifer shows a high vulnerability to pollution episodes. It discharges to the sea along the coastline and through pumping wells distributed along its entire surface.

There are many companies devoted to the manufacturing and manipulation of all kinds of oil-derivative products, many of which come into the category of light non-aqueous phase liquids (LNAPLs), e.g. petrol, and DNAPLs (e.g. chlorinated solvents), that began their activity in the 1960's. Various groundwater contaminant episodes with different types of hydrocarbons, such as naphthalene, have been detected since the 1970s.

The pollution episode studied was detected in 1996 at one industrial plant inside a chemical complex, but it is not known when the episode started. Two chlorinated methanes were associated with the episode: carbon tetrachloride (CT) and chloroform (CF) stored independently at the site. Repeated leaks and spills were responsible for it. After obtaining evidence of the existence of CT and CF in groundwaters and due to the sale of the plant's facilities to another chemical company, characterization studies, monitoring, and control of the groundwater quality began in 1996. Concentrations recorded at the groundwater, ranged between 15 and 22,600 µg/L for CF and between the detection limit and 86 µg/L for CT.

Since 1997, remedial works have been carried out consisting of initial gas extraction and injection of water vapor followed by a pump & treat (air-stripping) system. Despite the substantial reduction observed in groundwater concentrations between 1997 and 2006, pollution continues to be above acceptable values.

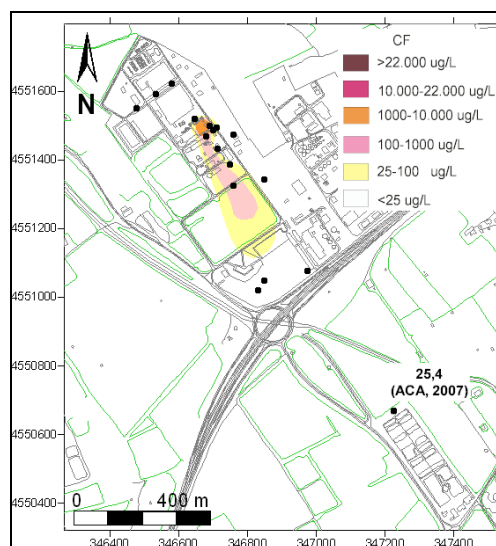


Figure 8. CF plume emerging from the source area in October 2008.

In April 2008, our team carried out a transect, consisting on two boreholes located 220 m downstream of the pollution source. The distance from one borehole to the other was 2.5 m. The recovery of the continuous cores made possible to carry out a soil and subsoil sampling at centimetric scale (65 and 71 samples were obtained respectively).

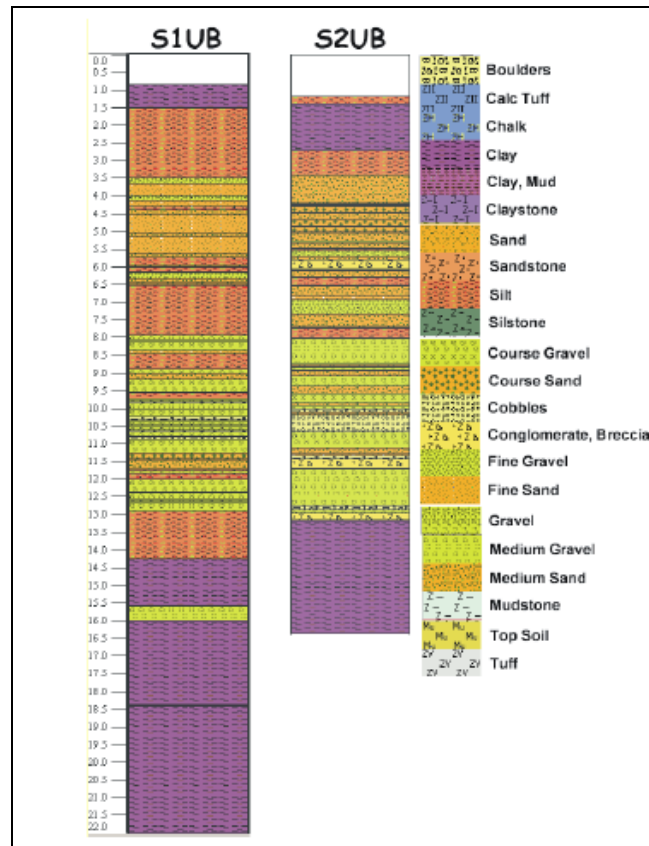


Figure 9. Geological characterization of the cores.

Table 3. Physical-chemical parameters determined at core samples.

S1UB	pH	CE ($\mu\text{S}/\text{cm}$)	Eh (mV)	C_T (ppm)	N_T (ppm)	C_{org} (ppm)	Cd (ppm)	Cu (ppm)
Max	8.89	523	155	88.51	0.97	0.97	13.33	75
Min	8.07	107.9	128	0.46	0.09	0.14	3.33	5
Geom mean	8.65	183.51	138.76	44.8	0.22	0.32	7.18	12.22
Std Dev	0.17	76.67	8.53	21.18	0.12	0.17	2.76	11.29
S2UB	pH	CE ($\mu\text{S}/\text{cm}$)	Eh (mV)	CT (ppm)	NT (ppm)	C org (ppm)	Cd (ppm)	Cu (ppm)
Max	9.69	320		91.98	0.45	0.59		
Min	8.31	15.8		0.8	0.05	0.11		
Geom mean	9.3	142.02		46.29	0.19	0.28		
Std Dev	0.26	48.64		21.56	0.07	0.11		

Table 4. Chlorinated solvents determined at core samples.

	S1UB (µg/L)											
	Chloromethanes			Chloroethanes				Chloroethenes				
% Samples >DL	33,3	81,0	41,3	25,4	15,9	17,5	33,3	42,9	17,5	6,3	22,2	20,6
VOCs	CT	CF	DCM	1,1,1,2 PCA	1,1,2,2 PCA	1,1,1 TCA	1,1,2 TCA	PCE	TCE	1,1 DCE	tDCE	cDCE
Max	563,56	1468,48	5730,38	131,57	2258,95	78,83	98,47	139,37	78,63	15,28	152,49	24,92
Min	1,14	3,83	1,46	1,67	1,38	1,47	1,55	1,34	1,59	1,34	1,38	2,35
Geom. mean	11,32	87,75	31,55	8,59	5,36	5,12	7,93	11,31	5,57	4,16	5,64	4,79
Std. Dev.	113,27	250,64	1357,19	26,90	293,55	11,27	20,68	31,94	11,38	2,83	20,30	4,73
Geom. mean + 2 Std. Dev.	237,87	589,03	2745,92	62,39	592,46	27,65	49,30	75,19	28,34	9,82	46,24	14,24
	S2UB (µg/L)											
	Chloromethanes			Chloroethanes				Chloroethenes				
% Samples >DL	25,4	73,1	35,8	52,2	19,4	22,4	19,4	58,2	23,9	11,9	14,9	16,4
VOCs	CT	CF	DCM	1,1,1,2 PCA	1,1,2,2 PCA	1,1,1 TCA	1,1,2 TCA	PCE	TCE	1,1 DCE	tDCE	cDCE
Max	406,37	1286,29	9053,49	848,62	1782,11	98,91	214,93	996,73	101,06	50,61	72,12	52,35
Min	1,38	2,69	2,57	2,80	2,19	2,26	2,61	1,63	2,51	2,30	2,32	2,25
Geom. mean	7,32	122,69	23,51	19,96	6,19	6,29	6,98	20,25	6,76	5,14	5,58	5,19
Std. Dev.	60,93	340,31	1606,59	164,58	238,60	18,22	32,30	193,27	17,80	8,12	12,13	7,20
Geom. mean + 2 Std. Dev.	129,18	803,30	3236,70	349,11	483,40	42,73	71,58	406,78	42,35	21,39	29,84	19,60

The maximum concentrations of PCE and TCE in the pore spaces correspond to the values recorded at the S2UB borehole, with 997 and 101 mg/L respectively.

The average CT values were similar in both boreholes (S1UB and S2UB) – 11.32 and 7.32 mg/L respectively – while for CF, they range between 88 and 123 mg/L, corroborating the greater presence of circulating CF in dissolved forms, as a result of which an important part has penetrated the matrix by molecular diffusion. In the S1UB borehole, the maximum concentrations found for CT are between 8.2 and 8.64 m, between 9.3 and 10.18 m and at 11.43 m deep, coinciding with silty levels. In fact, the presence of CT in silty levels is also shown in the aquitard, where maximums are detected at 15.63 m deep, between 17 and 17.94 m and at 18.4 m depth. Based on the sampling in the continuous cores from the boreholes it has been possible to make a semi-quantitative estimate of the diversity and degree of development of the different bacterial communities present in the non saturated zone and in the aquifer environments. Denaturing Gradient Gel Electrophoresis (DGGE) (see Fig. 10) [42], a molecular fingerprinting method that separates polymerase chain reaction (PCR)-generated DNA products was applied on selected samples. The polymerase chain reaction of environmental DNA can generate templates of differing DNA sequence that represent many of the dominant microbial organisms without single

identifications. As observed for mineral elements, the two boreholes are also characterized by a large degree of spatial and vertical heterogeneities from the microbiological point of view (see Fig. 11).

The piezometric oscillation zone, the capillary zone and, above all, the zones with granulometric contrasts constitute ecotones in which levels with very fine granulometry show the greatest microbial diversity and often the more developed communities.

Chemicals biodegradation takes place not only at the scale of the dissolved fraction but also at the scale of the fraction retained in the finest, low hydraulic conductive, materials. The lower presence of CT in the S2UB borehole than in the S1UB can be attributed to a greater degradation rate due to a higher proportion of fine materials.



Figure 10. DGGE equipment.

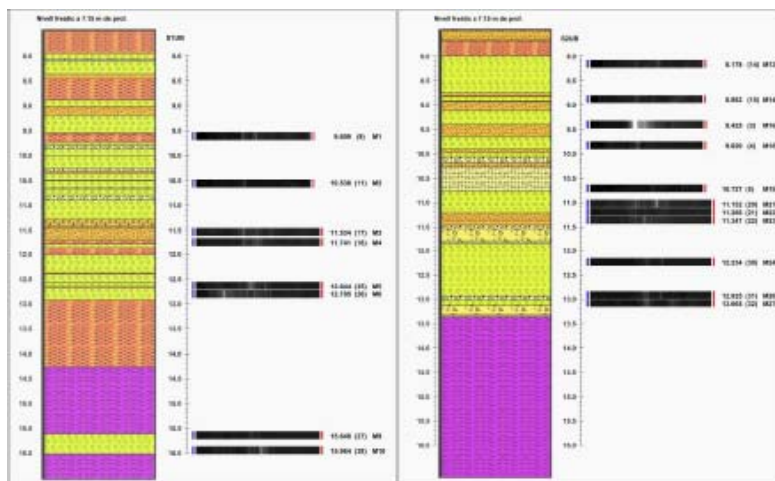


Figure 11. DGGE results in both sampled cores.

3. Remediation approach

Knowledge of chlorinated solvents sources concentration and half-life may be valuable in approximating time to cleanup, long-term monitoring plans, or meeting regulatory criteria for the sites.

Biodegradation processes of the lightest chlorinated solvents affect not only at the dissolved fraction but also at the fractions retained in the very fine, less conductive, materials. The quantification of this pollutant mass is one of the aspects that has been taken into account when designing remedial strategies in order to forecast back-diffusion and additional costs.

The proposed *in situ* remediation approach for both sites includes a combination of biostimulated microbial bioremediation by addition of hydrogen and nutrients [41], and rhizodegradation in the riparian zone in conjunction with monitored natural attenuation and an apatite barrier placed by infiltration, instead of by injection well, that allows sequestration of other bioavailable contaminants, such as metals, at the non saturated/vadose as well as at the saturated zone. Infiltration of apatite solution, made with an amorphous form of a carbonated hydroxy-apatite that has random nanocrystals of apatite embedded in it, from the soil surface extends the amount of time available for apatite emplacement and immobilizes contaminants in the vadose zone that would not be treated by the proposed techniques. Aluminum, present at site A, is effectively stabilized by apatite treatment.

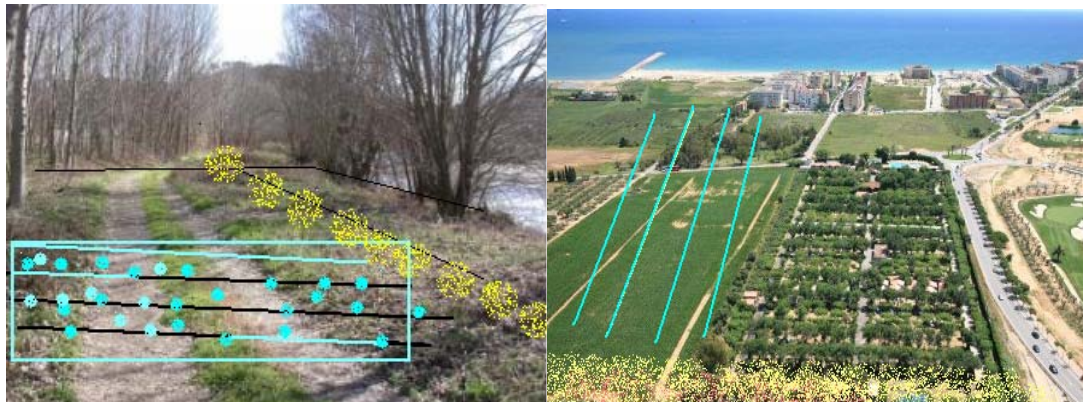


Figure 12. Proposed *in situ* remediation approach. (in blue groundwater flow; apatite infiltration is in yellow and precede phytoremediation areas).

Phytoremediation: Rhizodegradation

Partial restoration of soil quality with organic matter amendments is a prerequisite for successful phytoremediation. Such amendments are part of the strategies for diminishing the stresses that limit plant growth in the field.

Important considerations in plant species selection for rhizophytoremediation have included the following items: climatological requirements, tolerance levels with respect to chemicals, tolerance to drought-prone or poorly-drained conditions, depth of the root zone, growth rate, transpiration rate or water use, sensitivity to a wide variety of stressors (salts, calcium carbonate, fire,...), root exudates (specially phenolic compounds), deciduous or evergreen (for trees), native or non-native, maintenance requirements, commercial availability, and ecological relevance.

The more suitable tree species, according to our applied selection criteria, are: hybrid poplar (*Trichocarpa X deltoides*), eastern cottonwood (*Populus deltoides* Bartram ex Marsh) and loblolly pine (*Pinus taeda*). Hybrid poplar is already present in Site A. No pollution has been detected at their leaves but no data is available about pollutants' root concentrations.

Seedling quality improvement for planting in dry sites must be achieved before remediation starts.

Herbaceous plants can be also used for ground cover and erosion control but mowing is necessary; *Plantago lanceolata*, a very widespread species at Mediterranean polluted sites, can be useful but because of its use in tisanes and other herbal remedies in some villages, attention must be paid to prevent people entering the treated area.

The positive effect of arbuscular mycorrhiza in plant growth and nutrition has been well documented, however the effect of the symbiosis in the up-take of pollutants can vary depending on the fungal isolate, the plant and the contaminant/s concerned.

In a previous published work we have studied the effect of *Glomus intraradices* (BEG72) on the growth of *Plantago lanceolata* [43]. We have also tested the good conditions for arbuscular mycorrhiza in the preexisted hybrid poplar field.

Engineering genes, such as haloalkane dehalogenase (DhlA) and haloacid dehalogenase (DhlB), from bacteria that have the ability to dehalogenate a range of halogenated compounds, into high biomass producing plants is a possibility that is reduced nowadays to the laboratory scale.

The number of trees to plant, plant density, or mixtures to be backfilled into the tree holes are important considerations when planning phytoremediation.

Soil nutrients must be supplemented with care; fertilizers applied in slow-release form are recommended. Phosphorous is likely to be the most limiting nutrient in calcareous soils [44]; in these soils, hybrid poplars may respond well to addition of chelated zinc.

Actions can be simplified when using trees that can stand for 20 or 25 years at the sites.

4. Conclusion

Water chlorinated solvents biodegradation rate due to reductive dehalogenation determined in microcosm assays is consistent with the geochemistry of the sites.

A competition for electrons is established during degradation between chlorinated solvents, other organic pollutants, organic matter, and other electron acceptors that can be present at the media such as nitrates and sulphates.

Chlorinated solvents and other organic pollutants may also act as electron donors providing an energy source for certain microorganisms. With the continual exchange of electrons, redox chemistry is an important factor in chlorinated solvents biodegradation.

PCE, TCE, and CT generally require reducing conditions before they will transform to aliphatic compounds. Such conditions require the presence of enough organic substrate to reduce all of the oxygen (below 0,5 mg/L), nitrate (below 1 mg/L ideally), iron, and sulfate ideally (below 20 mg/L) before dechlorinating bacteria will successfully compete to reduce chlorinated aliphatic hydrocarbons.

In site A water nitrate concentration oscillates along the year between 5 and 200 mg/L, although concentrations of up to 525 mg/L may be reached in some dug well. Indicating that in spring or in some autumns biodegradation is not feasible at some points.

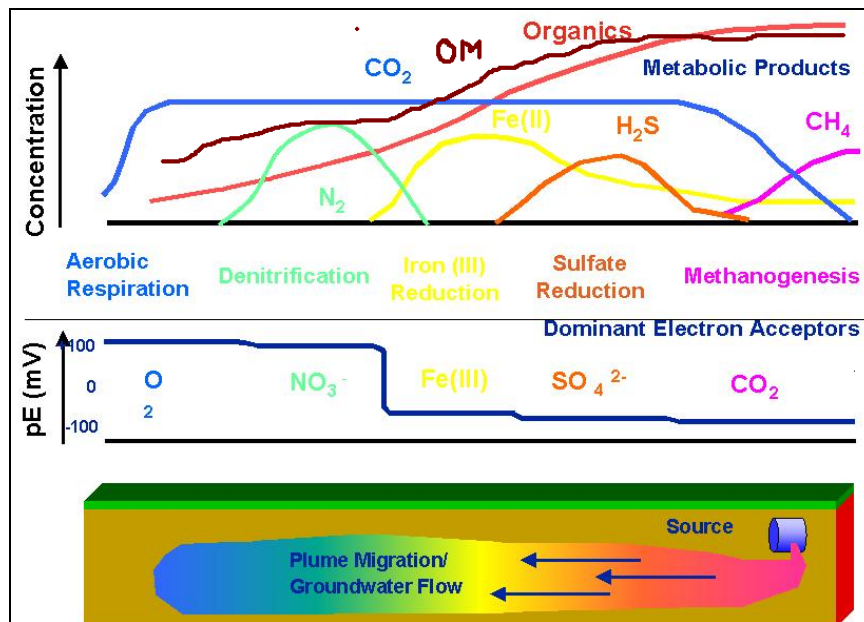


Figure 13. Organic matter and organics competition (a) and electron competition (b) schemes.

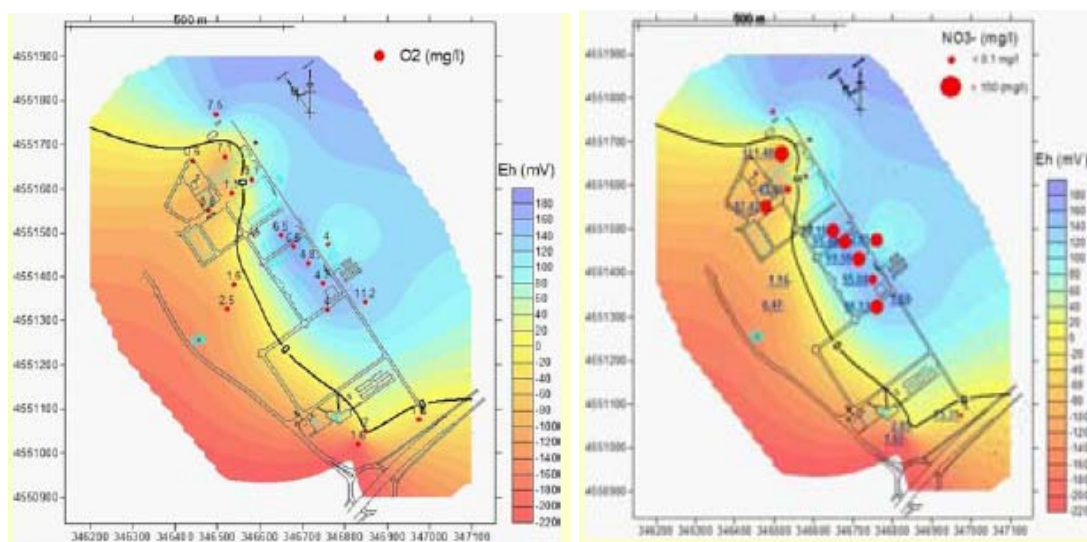


Figure 14. Oxygen and nitrates average concentrations at site B.

At site B (see Fig. 14) nitrate levels are lower, but still reach bad levels for reductive dechlorination. A previous water nitrate reduction treatment is needed in both sites.

The biodegradation of chlorinated solvents in soils, at low concentrations can be enhanced by using adapted mycorrhized trees producing phenolic exudates, but such biodegradation could be also affected by the ionic strength of nitrates or other salts when present.

Plants will also contribute to the remediation via chlorinated solvent uptake and dechlorination in plant systems.

Acknowledgements

The authors are grateful to Professor Beth Parker of the University of Guelph for her fruitful discussion. We would like to thank the Catalan Water Agency and staff members of some involved companies for the support and cooperation shown while carrying out the field work. We would also like to thank the members of the Department of Geochemistry, Petrology and Geological Prospecting of the University of Barcelona and, most particularly, the members of the Hydrogeology group. We would like to highlight the institutions financing the research carried out within the following research projects: CTM 2005-07824 and CGL 2008- 02164/BTE funded by the Spanish Ministry of Education and Science.

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