Use of compound-specific isotope analyses as a tool to demonstrate biodegradation of petroleum hydrocarbons in contaminated groundwater

Daniel Bouchard[1], Patrick Höhener[2] and Daniel Hunkeler[3]

[1]SNC-Lavalin Environnement Inc., Montréal, Canada. Email: daniel.bouchard@snclavalin.com
[2]Université de Provence, Marseille, France.
[3]Université de Neuchâtel, Neuchâtel, Suisse

Abstract

Natural attenuation of organic contaminants in groundwater was shown to be a cost effective strategy to manage petroleum hydrocarbon contaminated sites and the occurrence of biodegradation is often the key process to be demonstrated for a successful monitored natural attenuation program. To achieve such a task, it was shown that compound-specific isotope analysis is a reliable tool to assess biodegradation of organic contaminants dissolved in the groundwater, and more recently for gas-phase organic contaminants present in soil pores. This paper summarises the state-of-the-art application of stable isotope analyses by hydrogeologists to assess biodegradation of organic contaminants on field sites.

1. Introduction

The wide use of petroleum hydrocarbons as primary source of energy has unfortunately led to numerous spills of these non aqueous phase liquids (NAPLs) to soils. Over the past decade, natural attenuation has become a widely applied strategy to restore petroleum hydrocarbon contaminated sites and is increasingly accepted by regulators [1]. This strategy relies on the capacity of indigenous microorganisms to eliminate contaminants from the subsurface under natural conditions. This remedial strategy has become an attractive alternative to engineered remediation as it was shown to reduce the risk of impacts on man and the environment to an acceptable level with limited economical investments [2]. However, to make this strategy viable, it is imperative to demonstrate that biodegradation of hydrocarbons is actually occurring at the site. Microbial breakdown (i.e. biodegradation) is usually the only process to destroy the organic compound into CO_2 and clear evidence must be provided that the concentration depletion is linked to the microbial breakdown and not to physical (and non destructive) processes like sorption, dilution or dispersion. The dissociation of the biological process from the physical processes represents a real challenge for soil scientists and hydrogeologists when interpreting the changes in concentration. In this context, the use of stable isotope ratios of either carbon atoms (^{12}C and ^{13}C) or hydrogen atoms (^{1}H and ^{2}H) has proven its efficacy in providing reliable information on the fate of contaminants released in the environment [3].

This paper explains how hydrogeologists are taking advantage of a naturally occurring confection default of the atom in the domain of contaminated soil and groundwater. The review explains the state-of-the-art application of the isotope method on contaminated field sites to assess biodegradation of organic contaminants. Even if the method can be applied for a large panel of organic contaminants, this review however focuses on petroleum hydrocarbons assessed by carbon isotopes solely.

2. Dynamics of contaminant transport

Once the spill of NAPL has occurred, the NAPL migrates downward through the unsaturated zone resulting in a subsurface contamination. Due to interfacial tension, a fraction of the spilled NAPL is retained in the pores of the unsaturated zone and creates a long term source of contaminants from which volatile organic compounds (VOCs), such as benzene (B), toluene (T) and xylene (X) compounds are volatilizing for potentially long periods (Figure 1 A-B). Migration by vapour phase diffusion was shown to be the predominant transport process of VOCs under natural conditions and consequently creates a vapour phase contaminant plume in the soil gas, whereas biodegradation restrains the spreading of contaminants [4] [5]. The vapour phase diffusion is strongly influenced by the partitioning of VOCs between the gas, the water and the solid phase and by the geometry of the soil pores. Due to the diffusion process, migration of VOCs in the gas phase has been largely recognized as a mechanism for groundwater contamination even if the NAPL has not reached the water table [6].

In case of larger spills, the NAPL eventually reaches the surface of the groundwater. As petroleum hydrocarbons have a smaller density than water, the product (termed LNAPL) will tend to float on the groundwater and thus accumulate on its surface. In contrast, organic products such as chlorinated solvents have a larger density than water and consequently, the product (termed DNAPL) will tend to sink to the bottom of the aquifer. The latter case of contamination will not be reviewed herein. As groundwater flows underneath the zone of accumulated residual LNAPL, soluble compounds from the LNAPL will dissolve into the water and form an aqueous-phase plume (Figure 1 C-D). For gasoline products, the readily soluble components are mostly the BTX compounds. Once dissolved in the groundwater, the BTX compounds and other VOCs begin their lateral migration by advection with the groundwater flow. The advection process is the dominant transport of the compounds in the saturated zone, and diffusion process can be neglected. Once more, the migration of the compounds is strongly influenced by several factors. Therefore, the length and the width of the plume, or the spatial distribution of the contaminant concentrations, are governed by the constitution of the soil matrix (sand, silt and clay), by the geometry of the soil pores, by the heterogeneity of the system (impermeable clay lenses), by the partitioning of the compounds between the water and the solid phase (absorption onto organic matter), dilution (recharge by rain water), and by the vulnerability of compounds to biodegradation. Finally, the dissolved VOCs away from the source zone can also outgas from the groundwater and diffusing upwards in the soil air until reaching the soil surface, causing potential vapour intrusion of VOCs into houses.

If the management strategy of the contaminant plume is by natural attenuation, it becomes highly important to accurately predict the fate of the contaminant in the soil air or in the groundwater in order to assess health risks to communities in the area of the contaminated site or to evaluate the threat of a contamination of nearby public water wells.

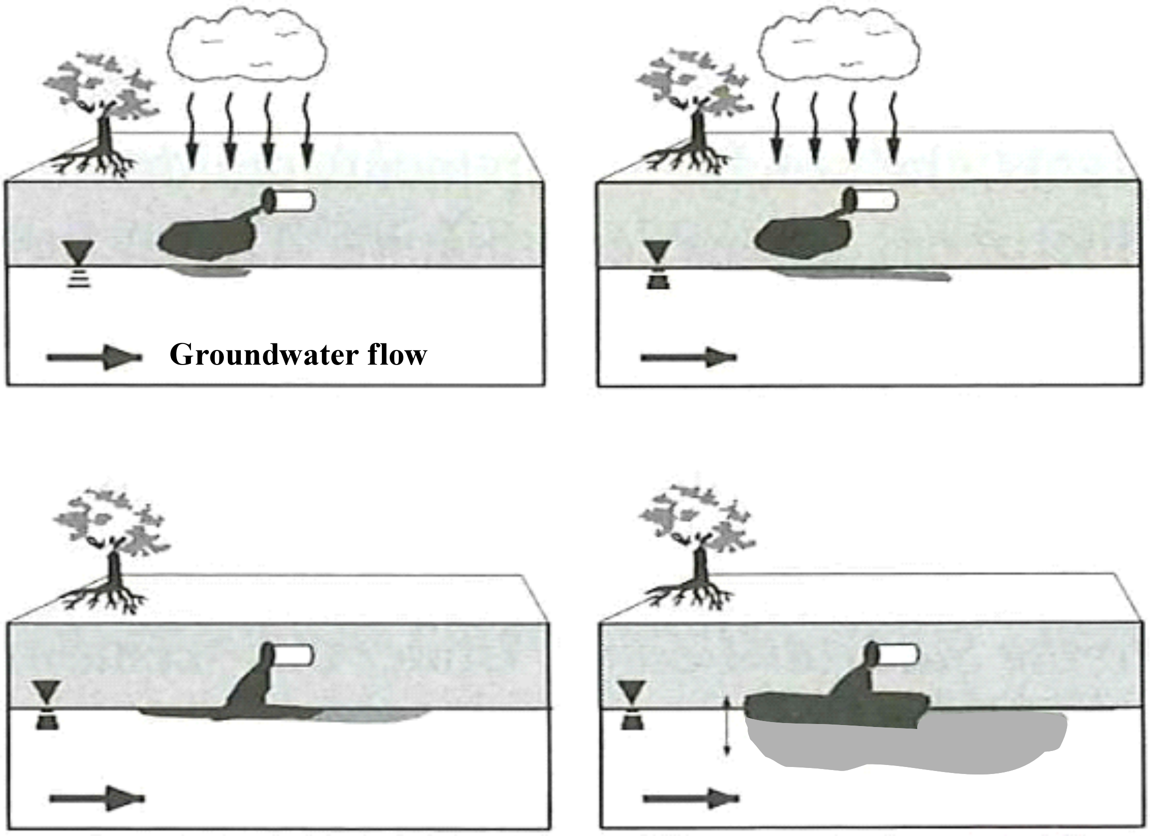


Figure 1: Contamination dynamics for a small petroleum hydrocarbon spill (A and B) and for a larger spill (C and D). Evolution of the plume over time is schematized from left (t=0) to right (t=x). Adapted from [7].

3. Stable isotopes in the environment

Heavy isotopes of carbon and hydrogen naturally occur in nature, and because petroleum hydrocarbons are mainly constituted by these two atoms, it is therefore expected to observe a heavy isotope in the constitution of some molecules. The mean global abundance of ^{13}C in nature is about one ^{13}C for 88 ^{12}C [8]. At this ratio, and as toluene is constituted of 7 carbons (Figure 2), we will find one molecule of toluene including a ^{13}C for about every 14 molecules of toluene encountered.

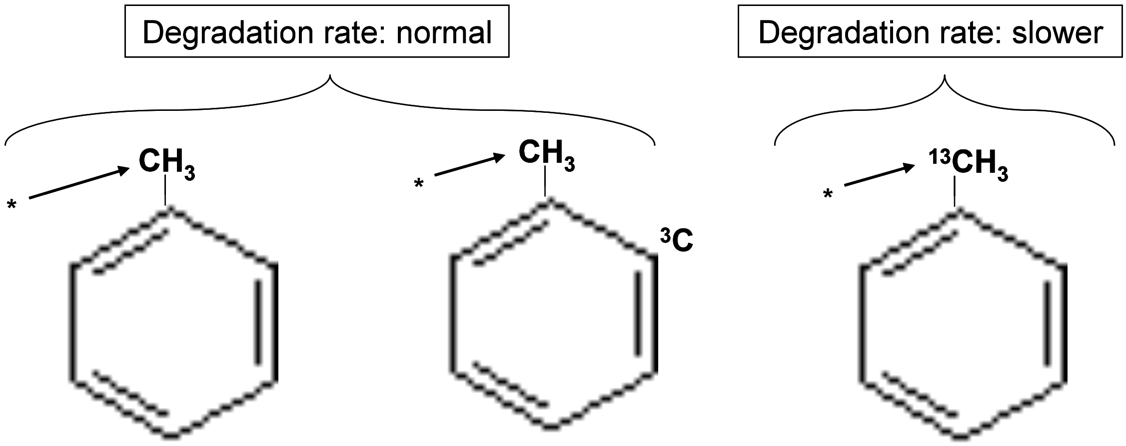
Usually, carbon isotope data are reported as isotope ratios in the δ notation relative to the VPDB standard (Peedee Belemnite, a belemnite fossil from the cretaceous Peedee formation in South Carolina) according to [9]:

$$\delta^{13}C\ (‰) = \left(\frac{R_{sample}}{R_{reference}} - 1\right)1000 \qquad (1)$$

where R_{sample} and $R_{reference}$ is the $^{13}C/^{12}C$ ratio of the measured sample and the reference material, respectively. A $^{13}C/^{12}C$ ratio (or $\delta^{13}C$, with units in ‰) for each compound of interest included in a mixture of contaminants can be quantified using the analytical system

Gas Chromatography-Combustion-Isotope Ratio Mass Spectroscopy (GC-C-IRMS). This isotopic method is termed Compound-Specific Isotope Analysis (or CSIA).

Every organic compound constituting a petroleum hydrocarbon source is expected to have its own $\delta^{13}C$ value, nevertheless contained in a range from -20‰ to -35‰ [10]. Because the synthesis of petroleum hydrocarbons is governed by time, the nature of deposited organic material, and the local geological conditions (such as the pressure), variation in these factors will confer a reservoir specific $\delta^{13}C$ value for each compound. Consequently, it is expected to observe a different $\delta^{13}C$ signature for the same compound originating from different geological reservoir. A substantial advantage of this non uniformity is that the $\delta^{13}C$ signature can serve as a fingerprint and may be used to differentiate 2 sources of contaminant on a same contaminated site.



*** = Location of the first enzymatic attack under anaerobic conditions**

Figure 2: Illustration of a toluene molecule and the importance of the location of the ^{13}C in the structure.

4. Principles of isotope fractionation induced by microbial activity

The presence of a supplemental neutron in the nucleus of an atom confers slightly different physical properties to the molecule containing that atom. The presence of a supplemental neutron does not create any additional electrical charges and thus the potential energy surface characterizing the chemical bond is invariable. However, the increase in nucleus mass directly affects the vibrational energy of molecules. The vibration frequency of a bond is given by [11]:

$$v = \frac{1}{2\pi}\sqrt{\frac{\kappa}{\mu}} \tag{2}$$

where v is the frequency of vibration of two atoms, κ is a constant force of the bond and μ is the reduced mass given by:

$$\mu = \frac{M_{w1}\,M_{w2}}{M_{w1} + M_{w2}} \tag{3}$$

where M_{w1} and M_{w2} are the different masses of the atoms involved. As the κ value is independent of the isotope and thus remains constant when either ^{12}C or ^{13}C is involved, only the change in mass can influence the frequency of vibration in equation 2. The frequency of vibration influences the level of free energy required for a reaction to proceed. Therefore, the small distinction in frequency of vibration between chemical species isotopically different is sufficient to create two different levels of activation energy required for the reaction to proceed. Because of the inverse relationship of v and μ, the heavier atomic mass of ^{13}C lowers the vibrational energy and hence increases the energy difference between product and transition state. In other words, chemical bonds between two light isotopes are more rapidly broken compared to bonds between a light and a heavy isotope.

In a more practical sense, hydrogeologists are expecting different biodegradation rates for molecules with light isotopes (^{12}C-molecules) compared to molecules containing one heavy isotope (^{13}C-molecules). The presence of a ^{13}C atom in the bond that is broken in the initial enzymatic transformation step can slightly slow down the biodegradation rate of the ^{13}C-molecules (see Figure 2). As a consequence, ^{13}C-molecules accumulate in the remaining contaminant pool over time. As illustrated in Figure 2, the position of the heavy isotope in the molecule is also important to create an isotope fractionation. The accumulation of ^{13}C-molecules in the remaining fraction is an univocal evidence of microbial activity degrading the contaminant as no other physical processes, such as those mentioned previously (dilution, dispersion, adsorption, etc), cannot cause significant isotope fractionation as no bond breaking reactions are involved.

5. Raleigh's Law and mathematical explanation of isotope fractionation

The magnitude of isotope fractionation between light and heavy molecules can be expressed by an isotope fractionation factor (α):

$$\alpha = {}^{h}k\,/\,{}^{l}k \tag{4}$$

where ${}^{h}k$ and ${}^{l}k$ stand for the biodegradation rate of the light and heavy molecule, respectively. The fractionation factor indicates the degree of separation of the isotope ratio between substrate and product and is usually less than one for bond-breaking reactions to indicate a ^{13}C enrichment in the substrate. The relationship between substrate concentration change and isotope fractionation allows quantifying the fractionation factor. The common equation that describes this progressive partitioning of the isotopic composition of the substrate is [9];

$$R = R_0 f^{(\alpha-1)} \tag{5}$$

where R and R_0 are the ratio and the initial ratio of heavy to light isotope of the contaminant, respectively, f is the remaining fraction of the contaminant. The derivation of equation 5 is based on the classical Rayleigh equation which describes fractional distillation of mixed liquids. Equation 5 is only valid for low abundance of the heavy isotope [12] and when the concentration decrease is solely caused by biodegradation. Even if advection process causes a decrease in VOCs concentration, the Rayleigh's law holds true as the flow rate of the groundwater is generally very slow [13]. However, in the case of diffusion of VOC in the unsaturated zone, the Rayleigh's law does not hold true, as the decrease of VOC caused by diffusion is considerable [14].

The fractionation factor is determined via microcosm experiments conducted in laboratory. For instance, Figure 3 shows laboratory results conducted on BTX. The results indicate a change in the isotope ratio linked to the decrease in concentration due to biodegradation. To date, fractionation factors for the most common contaminants are determined and can be taken from the literature. However, it must be known that the magnitude of the enrichment factors for a same compound can differ from site to site. Each type of microorganism creates different amplitude of accumulation as they have different enzymatic systems. Therefore, selection of the enrichment factor must be done carefully.

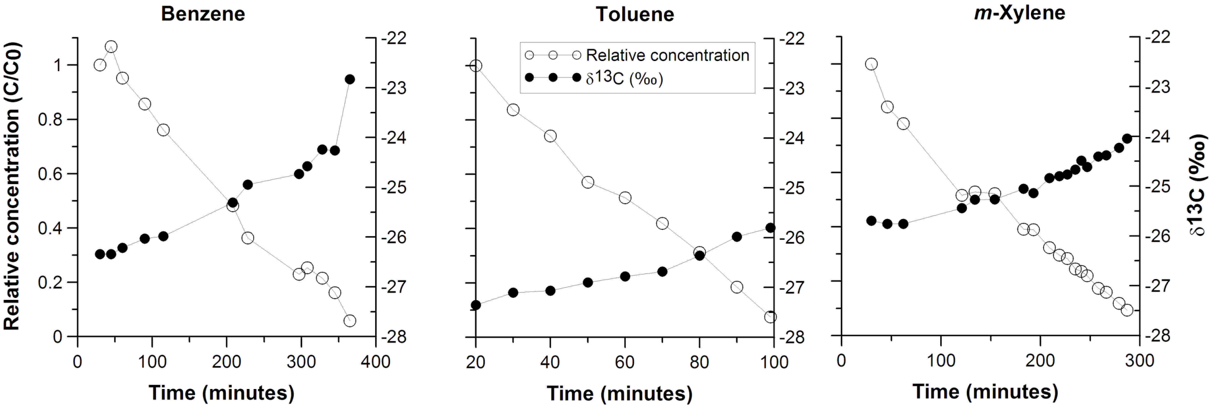


Figure 3: Relative concentration and evolution of the $\delta^{13}C$ values of the remaining substrate occurring over time during aerobic biodegradation of benzene, toluene and xylene, in unsaturated soil conditions. Adapted from [15]

6. Example of field application of the Compound-specific isotope analysis method

Compound-specific isotope analysis (CSIA) has been shown on several sites to be an effective tool to confirm *in situ* biodegradation by the indigenous microbial population. Therefore, the method is increasingly used to assess and sometimes to quantify *in situ* biodegradation of various types of organic contaminants located in the groundwater. Generally used in the saturated zone via water samples, the isotope method was recently shown to be applicable in the unsaturated zone via gas samples. Hence, the latest results fill a gap and allow a complete assessment of the fate of VOCs in both contaminated zones. A growing body of literature has described how isotope data can be used to assess and quantify biodegradation in the saturated zone of the subsurface (see more detailed reviews by [16], [17] and [18]. In the following section, a real case application of the CSIA method in the saturated zone is reviewed, and finally, a summary of the latest developments on the use of CSIA in the unsaturated zone and its implication is presented.

6.1 In the saturated zone

A complete CSIA assessment on a site contaminated by different organic compounds (volatile organic compounds and polycyclic aromatic hydrocarbons) was carried out by [19] to demonstrate *in situ* biodegradation of toluene dissolved in the groundwater. The site map is presented on Figure 4 and illustrates the groundwater flow direction, the investigated area and the location of the monitoring wells. The 15 selected monitoring wells for the assessment are located in a 110 m long transect along the groundwater flow path. The concentration assessment for toluene is illustrated in Figure 5 (left). The largest concentration of toluene was monitored in well B54 and thus can be considered to be the location of the source. Using concentration contour lines, Figure 5 illustrates the decrease in toluene concentration with increasing distance from the well B54. Based on these results, the contribution of biodegradation in decreasing the toluene content is a complicated task to demonstrate as several other factors must be taken into account. Such an evaluation can nevertheless be performed, but it requires the use of a numerical model which may quickly become time consuming.

On the same water samples used to determine the concentration, $\delta^{13}C$ measurements were also carried out. The results are presented in Figure 5 (center), using the same pattern as for concentration by addressing $\delta^{13}C$ based contour lines. Results show more positive $\delta^{13}C$ values in distance wells B28 and B55, compared to values observed in nearby wells B54 and B56 from the source area. These more positive values with increasing distance from the source indicate an accumulation of ^{13}C-toluene in the remaining toluene pool. This shift in $\delta\ ^{13}C$ values is a direct evidence that biodegradation is an active process that destroys toluene compounds as they migrate in the aquifer.

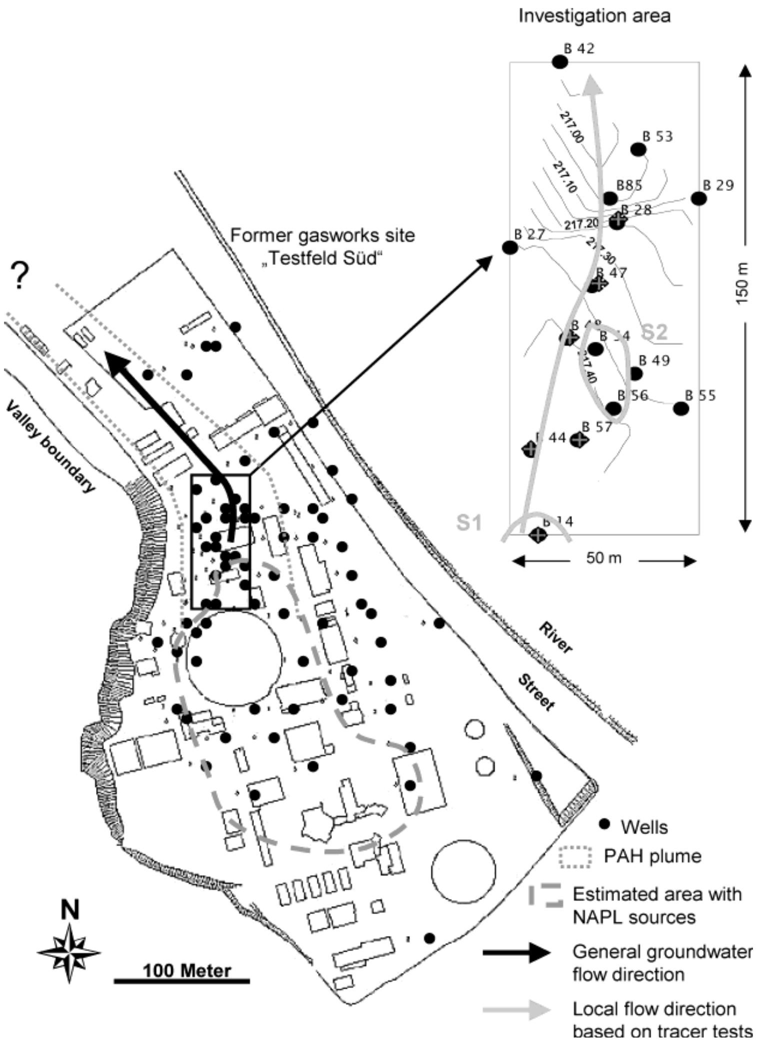


Figure 4: Site map of the petroleum hydrocarbon contaminated site (including BTX compounds and polycyclic aromatic compounds PAH). Adapted from [19]

Finally, by inserting $\delta^{13}C$ values in equation 5, quantification of the proportion of toluene that has been biodegraded is possible. Results are illustrated on Figure 5 (right) and indicate that more than 90% of the toluene input by the source has been biodegraded near wells B85, B29 and B55. Calculation of a biodegradation rate using $\delta^{13}C$ values is also possible, however it was not assessed in the frame of this study.

In conclusion, this isotopic characterisation of toluene has yielded scientific evidence that the compound is not being diluted, but is being destroyed during migration in the aquifer and the extent of the plume is restrained by microbial activities within the property limits.

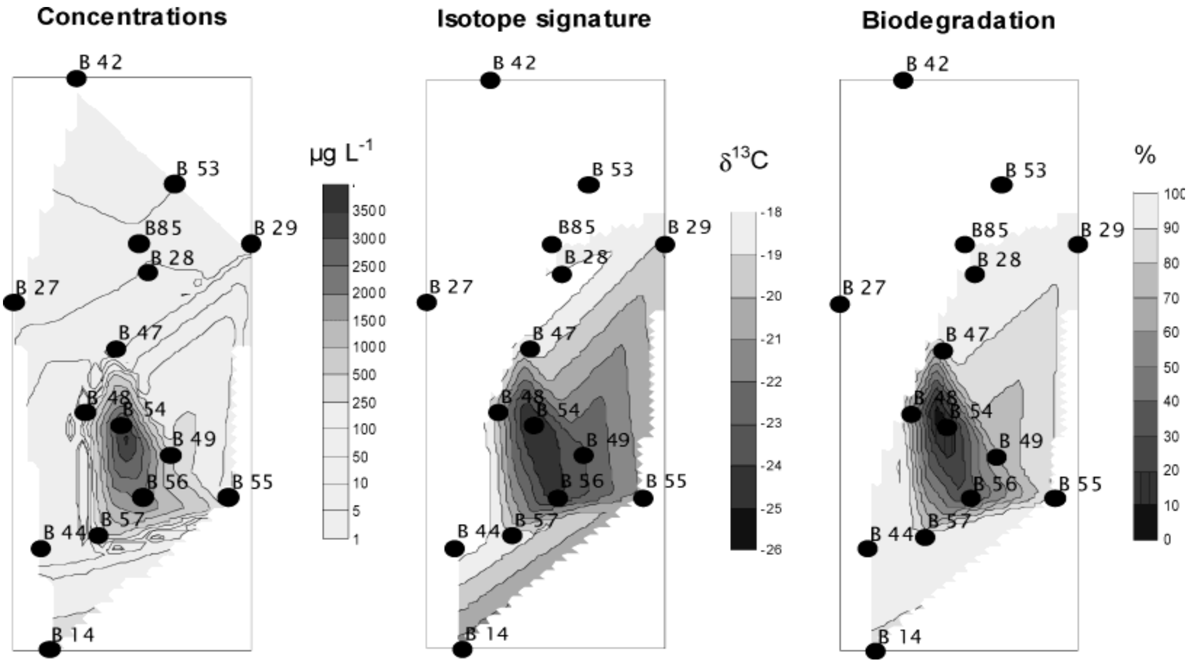


Figure 5: Illustration of the benzene concentration (left). Adapted from [19].

6.2 In the unsaturated zone

The isotope method has first been shown to be applicable in the unsaturated zone by only few studies, for both VOCs in the gas phase [20] or absorbed on soil sediments [21]. Recently, the feasibility of applying the isotope method has been investigated more thoroughly via soil air sampling. Measurements carried out during a laboratory experiment [22] and a field experiment [14] have allowed to better understand the mechanisms responsible of the isotope fractionation occurring during VOCs migration. Diffusion and biodegradation of VOCs lead obviously towards concentration decreases with increasing distance from the source, but also to an isotope fractionation. Mathematical reproduction of isotope ratios measured during the two previous experiments allowed to underline two processes controlling the isotope ratio. Beside biodegradation, a mass-specific diffusion coefficient based on the presence of a heavy isotope in the molecule creates an isotope fractionation. Molecules including a ^{13}C are diffusing slower than ^{12}C-containing molecules.

In order to better appreciate these implications, a theoretical investigation on $\delta^{13}C$ distribution of volatile organic compounds (VOCs) in a surrounding area of a point source contamination in the unsaturated zone was conducted [23] and is presented in Figure 6. The scenario deals with a large horizontal pool of light NAPL which has accumulated on the surface of the groundwater. The VOCs released from the contaminant source are diffusing upwards through the unsaturated zone where biodegradation takes place and, if biodegradation is incomplete, are lost to the atmosphere. The simulation reproduces the $\delta^{13}C$ of hexane in the gas-phase and diffusion is assumed to be under steady state conditions.

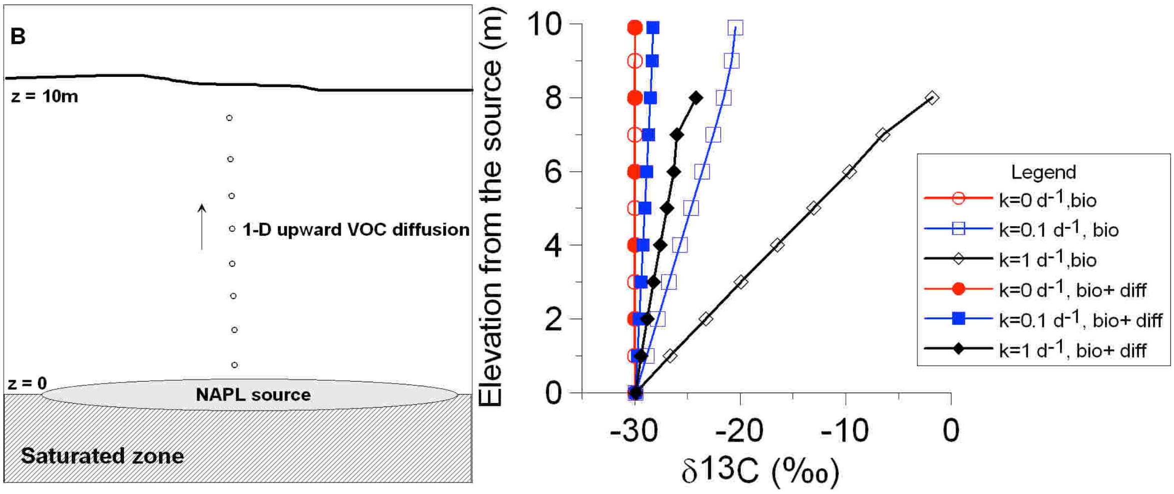


Figure 6: Mathematical simulation reproducing the $\delta^{13}C$ of hexane during upward migration through the soil air of the unsaturated zone, according to different biodegradation rates (0, 0.1 and 1 d^{-1}). Emphasis is given on the effect of diffusion causing an isotope fraction. Adapted from [23]

The results in Figure 6 indicate an effect of biodegradation on the isotope ratios, but also demonstrate the importance of the diffusion isotope effect. In absence of biodegradation (k=0), the simulations show no shift with distance (or depth), even if isotope-specific diffusion coefficients are used (i.e. ^{13}C-hexane are diffusing slower than ^{12}C-hexane). For the scenarios with biodegradation, inclusion of the diffusion isotope effect reduces the magnitude of the isotope shift with distance.

Compared to the saturated zone, the application of CSIA in the unsaturated zone is more demanding. A fractionation caused by an isotope-specific diffusion coefficient, which is not likely to occur in the saturated zone, must be taken into account jointly to biodegradation in order to interpret adequately the measured $\delta^{13}C$ values. The investigations performed allowed to draw significant conclusions about $\delta^{13}C$ behaviour in the unsaturated zone, both occurring in a system under steady state conditions. Firstly, a lack of biodegradation in a system can easily be identified since uniform $\delta^{13}C$ values with distance are expected even if ^{13}C-molecules migrate at lower velocity. Secondly, when biodegradation occurs, the flux of VOCs variations is solely due to biodegradation, but the related change in $\delta^{13}C$ is still controlled by biodegradation and diffusion. The change in $\delta^{13}C$ is linearly related with the change in flux, but an extended Rayleigh-type equation is required prior to make a quantitative assessment of the remaining fraction of the migrating VOCs [23].

Under the light of these results, new applications emerge for the use of stable isotopes analyses. For instance, using isotopes to assess vapor intrusion of petroleum hydrocarbons inside buildings in order to complement current tools. The isotope analyses will help understand the role of aerobic biodegradation in the attenuation of vapor [24]. Furthermore, stable isotope analysis can be used to establish a 3-point-connection between the VOCs measured in the air inside a building, the VOCs measured in the soil air underneath the building and the VOCs measured in the groundwater. The establishment of such a link provides the direct evidence required to identify the contributing source of the contaminant and to attribute responsibilities.

7. Summary and conclusions

This paper has reviewed how the use of carbon stable isotope analysis can be relevant in practice for hydrogeologists dealing with sites contaminated by organic products released by industrial activities. Stable isotope analysis of organic contaminants, such as petroleum hydrocarbons, but also of organic chlorinated solvents (dry cleaning), has evolved into a very promising method to demonstrate biodegradation of contaminants dissolved in the groundwater, and has newly been developed for gas-phase contaminants located in the soil air. The tool has been applied on several sites during the last decade and, as it has now established its reliability and robustness, it might become regularly included in the frame of monitored natural attenuation programs to scientifically document the fate of contaminants in the soil system.

As petroleum hydrocarbons are organic compounds biodegradable by indigenous soil microorganisms, controlled natural attenuation as remedial strategy becomes a valuable cost effective option. In order to demonstrate that natural attenuation is a viable and socially responsible solution, clear evidence that biodegradation decreases the mass of contaminant must be provided. In response to these needs, stable isotope analyses bring valuable information and:

- Provide information on the compound of interest mixed into a cocktail of contaminants.
- Allow to calculate the remaining proportion (in %) of the contaminant that has left the source area (assessment in the groundwater).
- Provide an unequivocal proof of microbial activity.
- Demonstrate a contaminant mass reduction caused by biodegradation
- Allow to quantify a biodegradation rate for the selected contaminant.
- Improve long term predictions of contaminant behaviours estimated by means of a numerical modelling.

In view to pursuit the development and the integration of stable isotope analyses in the field of contaminated sites, scientists are now evaluating the possibility to extend the method for larger organic contaminants (such as polycyclic aromatic compounds) using hydrogen or chlorine isotopes. In addition, scientists are also evaluating the possibility to take advantage of the presence of different isotopes in heavy metals, such as lead, mercury or copper, to differentiate the anthropogenic source of heavy metals contaminating the environment from naturally occurring high level content.

8. References

[1] Mulligan, C.N. and Yong, R.N., 2004. Natural attenuation of contaminated soils.

Environment International, 30: 587-601.

[2] NRC, 2000. Natural attenuation for Groundwater Remediation. National Academy Press, Washington DC, 274 pp.

[3] Aelion, M.C., Aravena, R., Höhener, P., Hunkeler D. (ED)., 2009. Environmental Isotopes in Biodegradation and Bioremediation". Tailor and Francis publishers, CRC Press. Boca Raton. 292-318.

[4] Baehr, A. L. and Corapcioglu, M.Y., 1987. A compositional multiphase model for groundwater contamination by petroleum products. 2. Numerical solution. Water Resources Research, 23 :201-213.

[5] Christophersen, M., Broholm, M.M., Mosbæk, H., Karapanagioti, H.K., Burganos, V.N. and Kjeldsen, P.2005. Transport of hydrocarbons from an emplaced fuel source experiment in the vadose zone at Airbase Værløse, Denmark. Journal of Contaminant Hydrology, 81: 1-33.

[6] Baehr, A.L., 1987. Selective transport of hydrocarbons in the unsaturated zone due to aqueous and vapor phase partitioning. Water Resources Research, 23: 1926-1938.

[7] Wiedemeier, T.H., Newell, C.J., Rifai, H.S. and Wilson, J.T., 1999. Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface. John Wiley & Sons, New York, 617 pp.

[8] IUPAC, 1991. Isotopic compositions of the elements. Pure and Applied Chemistry, 63: 991- 1002.

[9] Clark, I.D. and Fritz, P., 1997. Environmental isotopes in hydrogeology. Lewis Publishers, Boca Raton, Florida, USA, 328 pp.

[10] Murphy, B., Morrison, R., 2007. Introduction to Environmental Forensics. Elsevire(Pu). Academic Press. 776 p.

[11] Melander, L. and Saunders, W.H., 1980. Reaction rates of isotopic molecules. John Wiley, New York, 331 pp.

[12] Hunkeler, D., Meckenstock, R.U. and Richnow, H.H., 2002. Quantification of isotope fractionation in experiments with deuterium-labeled substrate. Applied and Environmental Microbiology, 68: 5202-5207.

[13] Abe, Y. and Hunkeler, D., 2006. Does the Rayleigh equation apply to evaluate field isotope data in contaminant hydrogeology? Environmental Science and Technology, 40 (5):1588-1596.

[14] Bouchard D, Hunkeler D, Gaganis P, Aravena R, Höhener P and Kjeldsen P. 2008. Carbon isotope fractionation during migration of petroleum hydrocarbon vapors in the unsaturated zone: field experiment at Værløse Airbase, Denmark, and modeling simulations. Environmental Science & Technology, 42(2); 596-601.

[15] Bouchard D, Hunkeler D and Höhener P. 2008. Carbon isotope fractionation during aerobic biodegradation of *n*-alkanes and aromatic compounds under unsaturated soil conditions. Organic Geochemistry, 39, 23-33.

[16] Elsner, M., Zwank, L., Hunkeler, D. and Schwarzenbach, R.P., 2005. A new concept to link observed stable isotope fractionation to degradation pathways of organic groundwater contaminants. Environmental Science and Technology, 39: 6896-6916.

[17] Meckenstock, R.U., Morasch, B., Griebler, C. and Richnow, H.H., 2004. Stable isotope

fractionation analysis as a tool to monitor biodegradation in contaminated aquifers. Journal of Contaminant Hydrology, 75: 215-255.

[18] Schmidt, T.C., Zwank, L., Elsner, M., Berg, M., Meckenstock, R U. and Haderlein, S.B., 2004. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. Anal Bioanal Chem, 378: 283-300.

[19] Griebler, C., Safinowski, M., Vieth, A., Richnow, H.H. and Meckenstock, R.U., 2004. Combined application of stable carbon isotope analysis and specific metabolites determination for assessing in situ degradation of aromatic hydrocarbons in a tar oil-contaminated aquifer. Environmental Science and Technology, 38: 617-631.

[20] Stehmeier, L.G., Francis, M.M., Jack, T.R., Diegor, E., Winsor, L. and Abrajano, T.A. jr., 1999. Field and in vitro evidence for in situ bioremediation using compound-specific $^{13}C/^{12}C$ ratio monitoring. Organic Geochemistry, 30: 821-833.

[21] Kirtland, B.C., Aelion, C.M. and Stone, P.A., 2005. Assessing in situ mineralization of recalcitrant organic compounds in vadose zone sediments using delta 13C and 14C measurements. Journal of Contaminant Hydrology, 76: 1-18.

[22] Bouchard D, Hunkeler D and Höhener P. 2008. Carbon isotope fractionation during volatilization of a petroleum hydrocarbon source and diffusion across a porous medium: a column experiment. Environmental Science & Technology, 42; 7801-7806.

[23] Bouchard D, Hunkeler D., Höhener P. Cornaton, F. “Analytical modelling of stable isotope fractionation of volatile organic compounds in the unsaturated zone”. Submitted to Journal of Contaminant Hydrology.

[24] Abreu, L.D.V., Johnson, P.C. 2006 Simulating the Effect of Aerobic Biodegradation on Soil Vapor Intrusion into Buildings: Influence of Degradation Rate, Source Concentration, and Depth. Environmental Science & Technology. 40 (7), 2304-2315