

Compound-specific isotope analysis for assessing sources and fate of aromatic hydrocarbons in contaminated aquifers

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Organic compounds deriving from industry, oil spills, improper disposal and/or leaking storage tanks, landfill leachates, household use, motor vehicle emissions as well as agricultural fertilizers and pesticides are responsible for widespread soil and aquifer pollution. The fate and behavior of such compounds in the subsurface depend on a number of physicochemical and biological processes, which may lead to 'natural attenuation'. Determination and quantification of these processes are crucial for contamination risk assessment and sustainable groundwater management. Compound-specific isotope analysis (CSIA) by online-coupling of capillary gas chromatography and isotope ratio mass spectrometry (GC-IRMS) offers a versatile tool to study the origin and to assess degradation processes of organic pollutants in the environment.

The aim of the present work is to demonstrate the potential of CSIA for studying sources and fate of aromatic hydrocarbons in aquifer systems. Headspace solid-phase micro extraction (hsSPME) was applied as sample pre-concentration and extraction technique allowing for compound-specific carbon and hydrogen isotope analysis ($\delta^{13}\text{C}$ and δD) of volatile compounds, even at concentrations in the low ppb-range. The analytical approach was thoroughly evaluated in terms of its precision, linearity and reproducibility.

Our study focuses on carbon and hydrogen isotope fractionation of aromatic hydrocarbons such as benzene and toluene (BTEX), and the possibility how to evaluate biodegradation processes *in situ* according to the Rayleigh-equation. Results obtained at sites with two different contamination scenarios will be presented: The first location is situated at a former coal carbonization plant; the second site represents a typical contamination due to a leaking underground storage tank at a fuel station. In addition to the possibility for assessing the extent of biodegradation of organic contaminants in soil and groundwater systems, we will demonstrate how isotope signatures can help revealing different pollution sources. Limitations and challenging aspects of the method will be critically addressed.

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Metabolic origin of $\delta^{13}\text{C}$ in dark-respired CO_2 : Comparison between leaves and roots

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The photosynthetic discrimination against ^{13}C leads to a ^{13}C -depletion of plant organic matter while the CO_2 left in the atmosphere gets ^{13}C -enriched. Thus, records of the variation of the isotopic composition of CO_2 above ecosystems are used to distinguish between photosynthetic and respiratory fluxes. However, the generally accepted hypothesis in such studies that no discrimination occur downstream photosynthetic fixation is now questioned. We have shown that the CO_2 respired by leaves in the dark is ^{13}C -enriched compared to organic matter, while it is ^{13}C -depleted in the case of roots. Although the relation between the metabolism and the ^{13}C of respired CO_2 has been explored in leaves, no such metabolic rationale is available for roots. To address this issue, we conducted ^{13}C -analysis on CO_2 and metabolites in typical conditions or in continuous darkness, both under natural abundance and following labelling with ^{13}C -enriched glucose or pyruvate (in specific carbon atom positions) using IRMS and NMR techniques. Surprisingly, the $\delta^{13}\text{C}$ of root-respired CO_2 remained constant under continuous darkness, despite the decrease in the respiration rate and respiratory quotient (RQ). Indeed, we have previously shown that the $\delta^{13}\text{C}$ of leaf-respired CO_2 decreased together with the decrease in RQ leading to a linear relationship between these 2 parameters. This strongly suggested that the variation in $\delta^{13}\text{C}$ is a consequence of a substrate switch to feed respiration: carbohydrate oxidation producing ^{13}C -enriched CO_2 and β -oxidation of fatty acids producing ^{13}C -depleted CO_2 compared to organic matter. This is consistent with the assumption that the $\delta^{13}\text{C}$ of dark-respired CO_2 in the leaves is determined by relative contribution of the 2 major decarboxylation processes in the dark: pyruvate dehydrogenase activity and Krebs cycle. Labelling on roots allowed us to infer, in typical conditions, an important contribution of the pentose phosphate pathway to respiration (22%). Continuous darkness mainly affected the Krebs cycle which seemed to become notably reduced. It is concluded that the invariance in the isotope composition of root respired CO_2 under continuous darkness is driven by compensations between both the different fractionating steps and the composition of the respiratory substrate mix.

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The use of stable isotopes for assessing Portuguese wine genuineness

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Wine is one of the most important beverages with an increasing role in international trading and economic income, being authenticity a key factor in establishing its effective cost.

The chemical composition of wine is affected by several factors including production area, grape variety, soil type, climate, oenological practices among others. These factors play an important role in discriminating wines according to their geographic origin and year of harvest, and in wine authentication as a proof of chaptalization, addition of water and sweetening with sugar.

By applying multivariate analysis techniques to stable isotope (^{13}C , ^{18}O , $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$) data obtained from authentic wines from several Portuguese grape growing areas, the influence of geographical origin, climate, year and date of vintage are discussed.

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Mytilus galloprovincialis
as a bioindicator of environmental conditions:
the case of the eastern coast of the Adriatic Sea

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The marine mussel (*Mytilus galloprovincialis*) lives attached to the surface of hard substrata, where its exposure and relative immobility allows it to record changes in ambient seawater. It is also found along the Eastern coast of the Adriatic Sea.

Oxygen and carbon isotopes were analyzed for calcite and aragonite in separate shell layers, while major, minor and trace elements in the bulk shell were analyzed to evaluate environmental conditions, such as the temperature of carbonate deposition, freshwater influence and locations of anthropogenic pollution. We found that, on average, aragonite is enriched by 1.1‰ in ^{13}C and by 0.2‰ in ^{18}O compared to calcite. The calculated temperatures for *M. galloprovincialis* shell growth from the investigated area range from 13.4 – 20.9°C for calcite and 16.6 – 23.1°C for aragonite. According to the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of shell layers, we can separate the investigated area into three locations: those with more influence of fresh water, those with less influence of fresh water and those with marine environments. The highest concentrations of manganese, barium, boron, arsenic, nickel, chromium were observed in shells from Omiš, Bačvice, Zablace (Central Adriatic) and Sv. Ivan (South Adriatic), where chemical and heavy industries are located, and where sewage is known to be discharged into coastal areas. The highest concentrations of zinc, lead and copper were measured in samples from Pula, Rijeka and Gruz, where there are also ports in addition to industry.

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Tracing biogeochemical processes and pollution sources with stable isotopes in river systems: Kamniška Bistrica (Slovenia) case study

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The geochemical study of river water allows important information to be obtained on chemical weathering of rocks/soil and to determine biogeochemical processes in water. Few studies have attempted to identify sources of nitrate in surface water using stable isotopic ratios.

The River Kamniška Bistrica has an HCO_3^- - Ca^{2+} - Mg^{2+} type water. Its $\text{Ca}^{2+}/\text{Mg}^{2+}$ molar ratio indicates that calcite weathering prevails in the watershed. The River Kamniška Bistrica and its tributaries are oversaturated with respect to calcite and dolomite. The pCO_2 pressure is on average up to 4 times over atmospheric pressure and represents a source of CO_2 to the atmosphere. $\delta^{18}\text{O}$ values of river water indicate primary control from precipitation and enrichment of the heavy oxygen isotope of infiltrating water recharging the Kamniška Bistrica from its slopes. The $\delta^{13}\text{C}_{\text{DIC}}$ values range from -3.6‰ to -10.7‰ and are controlled by biogeochemical processes in terrestrial environments and in stream: (1) exchange with atmospheric CO_2 , (2) degradation of organic matter, (3) dissolution of carbonates, and (4) tributaries. The most important process in the upper flow of the river is carbonate dissolution, and combination of carbonate dissolution and organic degradation in lower flow. The most important contributions will be further calculated in detail according to steady-state equations at the mouth of the river. $\delta^{15}\text{N}$ ranges from -1.9‰ (source) to 8.0‰ (lower flow) in autumn sampling season and from -5.2‰ (source) to 4.5‰ (lower flow) in winter sampling season, respectively. Higher $\delta^{15}\text{N}$ values in the lower flow of the river indicate anthropogenic pollution activity.

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Application of geochemical and isotopic analysis and their role in the examination of coal gas genesis

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Coal gas is the gas of various origins that resides in the coal structure. It is composed of CO₂, methane, nitrogen, higher hydrocarbons (ethane, propane, butane). In general three main sources of gases can be distinguished: 1) abiogenic, 2) biogenic and 3) thermogenic. During migration, the gases of different origin may mix.

The major components of coal gases in the Velenje lignite seam are CO₂, methane and nitrogen. Concentrations of CO₂ vary from 18 to 98.8%, of methane from 1.1 to 100%, and of nitrogen from 7.2 to 67.3%. Ratio between CO₂ and CH₄ concentrations varies over a wide range and is crucial at higher values. Variations of concentration and isotopic composition indicate various sources of coal gases with possible mixing and other physico-chemical processes during migration, as diffusion and sorption.

During mining of lignite in the Velenje coalmine drastic changes of all measured gas parameters occur. At each working face short and long boreholes are placed to monitor gas concentrations and isotopic composition as well as mechanical parameters with the distance of the working face. Especially important is the drop of concentration of excess nitrogen with approaching of the working face – the excess nitrogen being formed in the coal seam. Change of two typical values of $\delta^{13}\text{C}_{\text{CH}_4}$ during approaching of working face toward boreholes indicates the emptying of at least two reservoirs. The utmost positive $\delta^{13}\text{C}_{\text{CO}_2}$ values (-5‰) and the utmost negative $\delta^{13}\text{C}_{\text{CH}_4}$ values (-72.8‰) were measured when the working face was already far ahead. Monitoring of coal gases in Velenje coalmine at working faces is important since beside other measured parameters, e.g. petrological, geomechanical and structural, can assure safety and eventually prevent sudden coal and gas outbursts.

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Carbon and hydrogen isotopic variations in a low pressure methane-poor hydrocarbons deposit

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In order to increase recovery of gas and oil from deposits technologies called Enhanced Gas Recovery with injection of CO₂ are used. In this process isotopic effects are expected. Therefore we have analysed isotopic composition of carbon and hydrogen in methane in one EGR object.

Carbon and hydrogen stable isotopic observations of methane from the EGR object have been carried out since September 2009. Gas samples (n=60) have been prepared manually on the lines, where the methane was separated from other carbohydrate gases by cryogenic purification using molecular sieves, a dry ice/ethanol mixture, and liquid nitrogen. The methane was passed through a copper oxide furnace (850°), where it was completely combusted to CO₂ and H₂O, which were then separated cryogenically. Carbon stable isotopic composition of methane was analysed off-line on dual inlet system (Finnigan MAT delta E IRMS) and hydrogen stable isotope composition of methane was analysed off-line on Delta V Advantage IRMS.

Isotopic composition of carbon in methane varied from -35.05 to -36.76‰ and isotopic composition of hydrogen in that methane varied from -96.5 to -134.7‰. Both spatial and temporal variations in carbon and hydrogen isotopic ratios in methane show that the system is very dynamic. It has been observed clearly that there are some preferential paths of methane transportation through the deposit, what resulted in isotopic effects. Namely, the carbon and hydrogen isotopic ratios show that the front of the highest velocity of methane movement is marked by the lowest isotopic ratios.

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Bone collagen in mammals from the outskirts of the Early Roman Empire

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Stable isotopes from bone collagen are a valuable and widely utilized tool in reconstructing paleodiets of human and animal populations as $\delta^{13}\text{C}$ in bone collagen is controlled by the type of diet (e.g. C_3 vs. C_4 plants), while $\delta^{15}\text{N}$ is constrained by the trophic level. Little such work has been carried out on samples from the outskirts of the Early Roman Empire.

Artifacts collected at the Ardeu site (SE limb of the Apuseni Mountains, Romanian Western Carpathians) indicate that this settlement was inhabited from the Eneolithic to the Bronze Age, during the Dacian Kingdom, and through the Middle Ages. The mammalian tooth samples used for collagen extraction were found along with items dating back to the Dacian Kingdom, centuries I BC–I AD.

The values of $\delta^{13}\text{C}$ of collagen ($n=21$) range between -21.9 and -17.1‰ (PDB), while $\delta^{15}\text{N}$ vary between 3.9 and 8.5‰ (AIR). Jaw bone and tooth root from a single individual yielded $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in close agreement. The teeth belonged to omnivores (*Suus*) and herbivores (*Equus*, *Ovis*, *Capra*, *Bos* and *Cervus*) bred or hunted by Dacians. Isotope data show no clear patterns of distribution, which could indicate either frequent changes in agricultural/grazing habits over a few centuries, or the effects of seasonal transhumance on the animals' diet. The majority of plants in the Dacian Kingdom 2 Ky ago belonged to the C_3 (e.g. barley, legumes) group, with few C_4 staple crops (e.g. sorghum and millet), which explains the variation in $\delta^{13}\text{C}$ values.

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Deuterium depletion as a new approach in cancer treatment and prevention

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It is known that the deuterium/hydrogen (D/H) mass ratio is the largest of stable isotopes of the same element, causing differences in the physical and chemical behaviour between the two hydrogen isotopes. Although the concentration of D is more than 10 mM in living organisms the possible role of D had not been investigated for 6 decades after its discovery in the early 30s.

In order to investigate the possible role of naturally occurring D in living organisms, in cell growth, tumor development and prevention, D-depleted water (DDW) was used. DDW caused tumor regression in xenotransplanted mice (MDA and MCF-7, human breast; PC-3, human prostate) and induced apoptosis *in vitro* and *in vivo*. Deuterium depletion inhibited the expression of certain genes (c-myc, H-ras, Bcl-2, K-Ras, COX-2) having key role in tumor development and prevented the appearance of cancer in mice after tumor induction with DMBA. During the four-month-long DDW administration in the phase II, double blind clinical trial, 7 out of 22 prostate cancer patients achieved partial response (PR), while only one patient out of 22 showed PR in the control group ($p=0.027$). In the treated group, net decrease in the prostate volume was three times higher (160.3 cm^3 vs. 54.0 cm^3 , $p=0.0019$). The one year survival was significantly higher in patients treated with DDW (logrank test, $p = 0.029$). The mortality rate decreased substantially in the treated group by the end of the first year ($p=0.034$).

In a retrospective study with breast cancer patients the relapse rate, within five years after the tumor was removed by surgery, was reduced to 22% from the expected 50%.

We suggest that cells are able to regulate D/H ratio and its changes can trigger molecular mechanisms having key role in cell cycle regulation. The decrease in D-concentration can intervene in the signal transduction pathways thus leading to tumor regression.

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Tracing the stable isotope composition of oxygen and hydrogen in precipitation from tree ring cellulose – examples from an oak and two pine tree sites in Finland

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Records of oxygen and hydrogen isotope ratios in tree rings are used to trace past environmental and climate conditions. Change in the isotope composition of precipitation, source water for trees, is connected to atmospheric air mass circulations, and is known to correlate with the temperature at the precipitation site for mid- and high latitudes.

We have studied the relationships between $\delta^{18}\text{O}$ ($\delta^2\text{H}$) values in tree ring α -cellulose (nitrated cellulose) and precipitation at three sites for the period 1989–2002. Pines (*Pinus sylvestris*) are from northern and eastern Finland and oak (*Quercus robur*) from southern Finland.

The $\delta^{18}\text{O}$ signal in tree ring cellulose has the highest correlation with summer (monthly average JJA) precipitation for oaks in southern and with previous year autumn (SON) precipitation for pines in eastern Finland. A lower correlation is obtained for pines in northern Finland.

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Organic matter sources in sediments of Szczecin Lagoon (NW Poland) indicated by C and N isotopic composition

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The C/N and stable C and N isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of sedimentary organic matter (OM) were determined in the Szczecin Lagoon – the area of the Odra River discharge into the Baltic Sea –, where gradual mixing of riverine and seawater occurs.

Organic matter content in sediments achieved maximum value in central part of the lagoon (22%) and decreased closer to the shore (0.2%). Carbon isotopic composition of OM ranged from -29.4‰ in central part to -26.4‰ in the mouth of the Dziwna river channel. $\delta^{15}\text{N}$ varied between 5.6‰ in the central area to 12.9‰ near the shore, while the C/N was changing in a narrow range from 9.7 to 11.9. $\delta^{13}\text{C}$ and C/N ratio of riverine suspended OM from Odra river ($\delta^{13}\text{C} = -30.6\text{‰}$; C/N = 9.6) and cyanobacteria ($\delta^{13}\text{C} = -30.2\text{‰}$; C/N=9.4) from the central part of the lagoon were characteristic for freshwater phytoplankton, however in the areas of seawater inflow contribution of marine algae to suspended organic matter was proved by OM enrichment in ^{13}C ($\delta^{13}\text{C} = -25.8\text{‰}$; C/N = 9.2).

Significant correlation have been found between $\delta^{13}\text{C}$ and OM content ($r = -0.54$, $p = 0.002$) as well as between $\delta^{15}\text{N}$ and both: organic nitrogen content ($r = 0.65$, $p = 0.012$) and organic carbon content ($r = 0.70$, $p = 0.006$) in sediments.

Our data indicate that OM in sediments of the lagoon is originated mostly from freshwater algae and cyanobacterial cells ($\delta^{13}\text{C} \sim -29\text{‰}$; $\delta^{15}\text{N} \sim 10\text{‰}$, C/N ~ 10). In the area of seawater inflow carbon isotopic composition of sedimentary OM was somewhat changed due to contribution of marine algae ($\delta^{13}\text{C} \sim -27\text{‰}$; $\delta^{15}\text{N} \sim 10\text{‰}$, C/N ~ 10). Occurrence of terrestrial OM in sediments was not confirmed.

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The carbon isotopic composition of dried and carbonized plant samples

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Monitoring ^{14}C activity in plant samples in the vicinity of the Nuclear power plant Krško (NPPK) is performed regularly since 2006 to estimate the possible influence on environmental ^{14}C levels and the possible contribution to the effective dose of local population through food chain. When reporting ^{14}C activity (Bqkg^{-1}C) of the samples, it is necessary to consider the stable isotopic composition of carbon ($\delta^{13}\text{C}$) and the fractionation that occurs during photosynthesis. In addition, isotopic fractionation can occur also during sample preparation. Therefore, the aim of our study was to analyse selected C3 and C4 plant samples in order to (1) determine their actual $\delta^{13}\text{C}$ values to be used for correction of ^{14}C activity and (2) investigate eventual isotopic fractionation which might occur during sample carbonization process, i.e. pyrolysis by analysing dried and carbonized counterparts of selected plant samples.

In this study $\delta^{13}\text{C}$ of 37 dried and 34 carbonized samples of apples, maize (leaves and grain) and wheat collected in four sampling campaigns (2008 and 2009) in the vicinity of NPPK was determined. Carbonized samples were ground to powder and homogenized. The dried samples were ground using different grinding methods. Apple samples were ground to powder and homogenized in an agate mortar. Maize and wheat samples were lyophilized after being frozen in liquid nitrogen. Four lyophilized samples were ground in a mill. During this process it was not possible to obtain completely homogenized samples, therefore, the rest of maize and wheat samples were ground and homogenized in an agate mortar. $\delta^{13}\text{C}$ was determined using a Europa 20–20 isotope ratio mass spectrometer (IRMS). The mean $\delta^{13}\text{C}$ value for analysed C3 plant samples (i.e. apple and wheat) and for C4 plant samples (i.e. maize) was close to -27‰ and -12‰ , respectively. In wheat, apple and maize samples, no significant differences (at $p < 0.05$) were observed between the dried and carbonized samples, however, an up to 2.2‰ and 1.8‰ difference was observed for particular apple and maize leaves samples, respectively. The influence of variability of $\delta^{13}\text{C}$ values on reported ^{14}C activity will be discussed.

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