

Compound-specific stable isotope analysis (CSIA) to model organic contaminants in catchment.

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Current Global Environmental Challenges

A pair of hands is shown holding a small, colorful globe of the Earth. The globe is positioned in the center of the image, with the hands cupping it from the sides. The globe shows continents in green and yellow and oceans in blue. The background is a dark, blurred brown.

1. Climate change

2. Global warming

3. Extreme weather events

4. Deforestation

5. Air pollution

6. Biodiversity loss

7. Water pollution

8. Water scarcity

Closely Correlated

Organic Water Pollutants

Water scarcity and water pollution are strongly connected, as pollution reduces the accessibility of clean and safe water.

The organic pollutants present in aquifers and catchments are:



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graph TD; A[Organic Water Pollutants] --> B[Legacy Contaminants]; A --> C[Micropollutants]; B --> D[Contaminants from past human activities that persist in the environment for long periods, even after their use has been reduced or banned.]; C --> E[Contaminants that are present in water at very low concentrations but can still have significant effects on human health and the environment.];
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Legacy Contaminants



Contaminants from past human activities that persist in the environment for long periods, even after their use has been reduced or banned.

Micropollutants



Contaminants that are present in water at very low concentrations but can still have significant effects on human health and the environment.

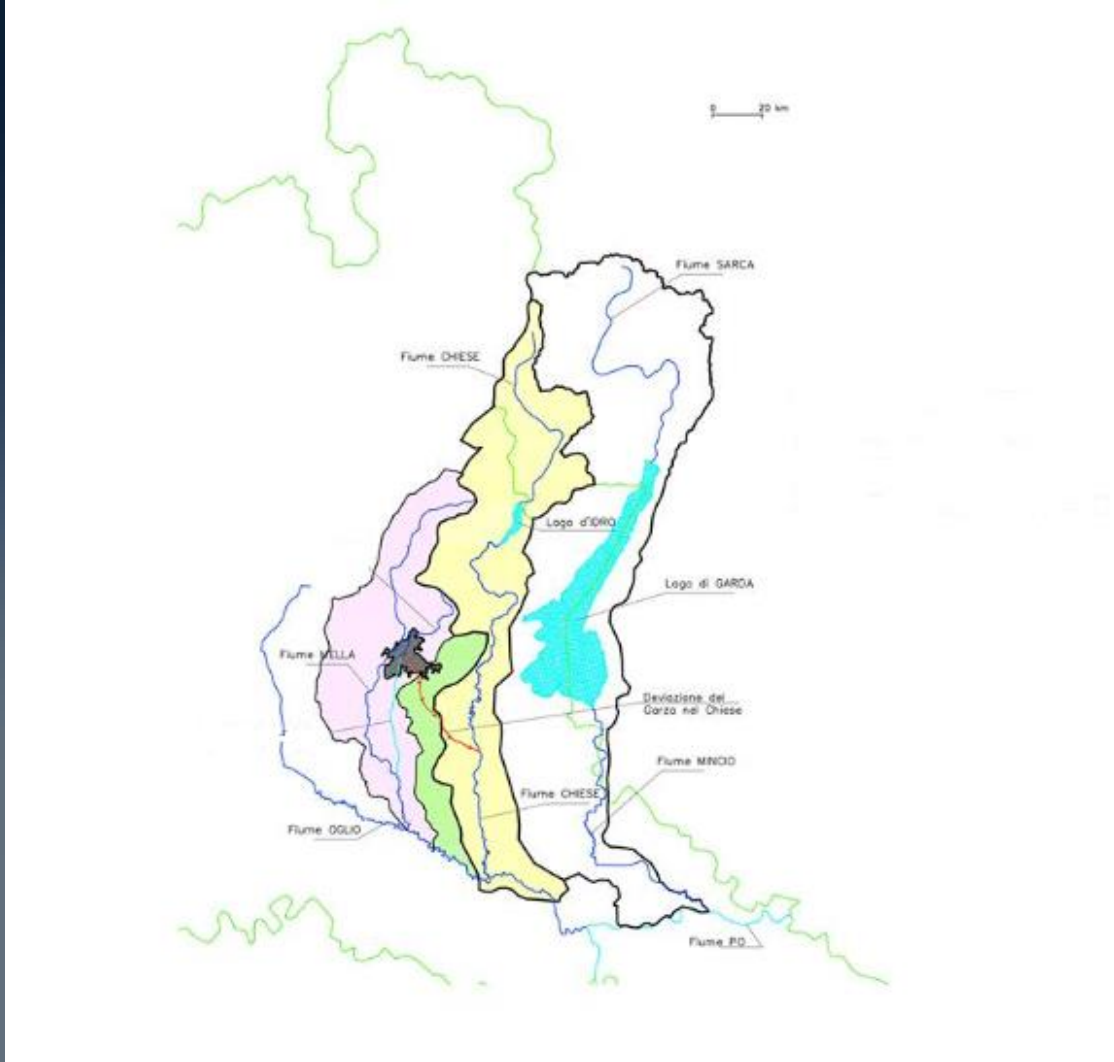
Mantova case: a project between Unipr and Unicatt

Rivers monitored in 2025: Chiese, Oglio, Mincio, and Mella



Banned pesticides found:

- HCH, HCH-beta, HCH-gamma (banned in 2007)
- Atrazine (banned in 1992)
- Bentazone (banned in 2023)
- Imidacloprid (banned in 2018)
- Metolachlor (banned in 2024)
- Chlorpyrifos-methyl (banned in 2020)
- Flufenacet (banned in 2025)



Organic Water Pollutants: Mantova case

Banned pesticides detected:

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Have they undergo degradation over time?

Monitor their metabolites (if they have)

If the pesticide has not been metabolized, or if its metabolites are not detected...

Banned pesticides and Compound-Specific Stable Isotope Analysis (CSIA)

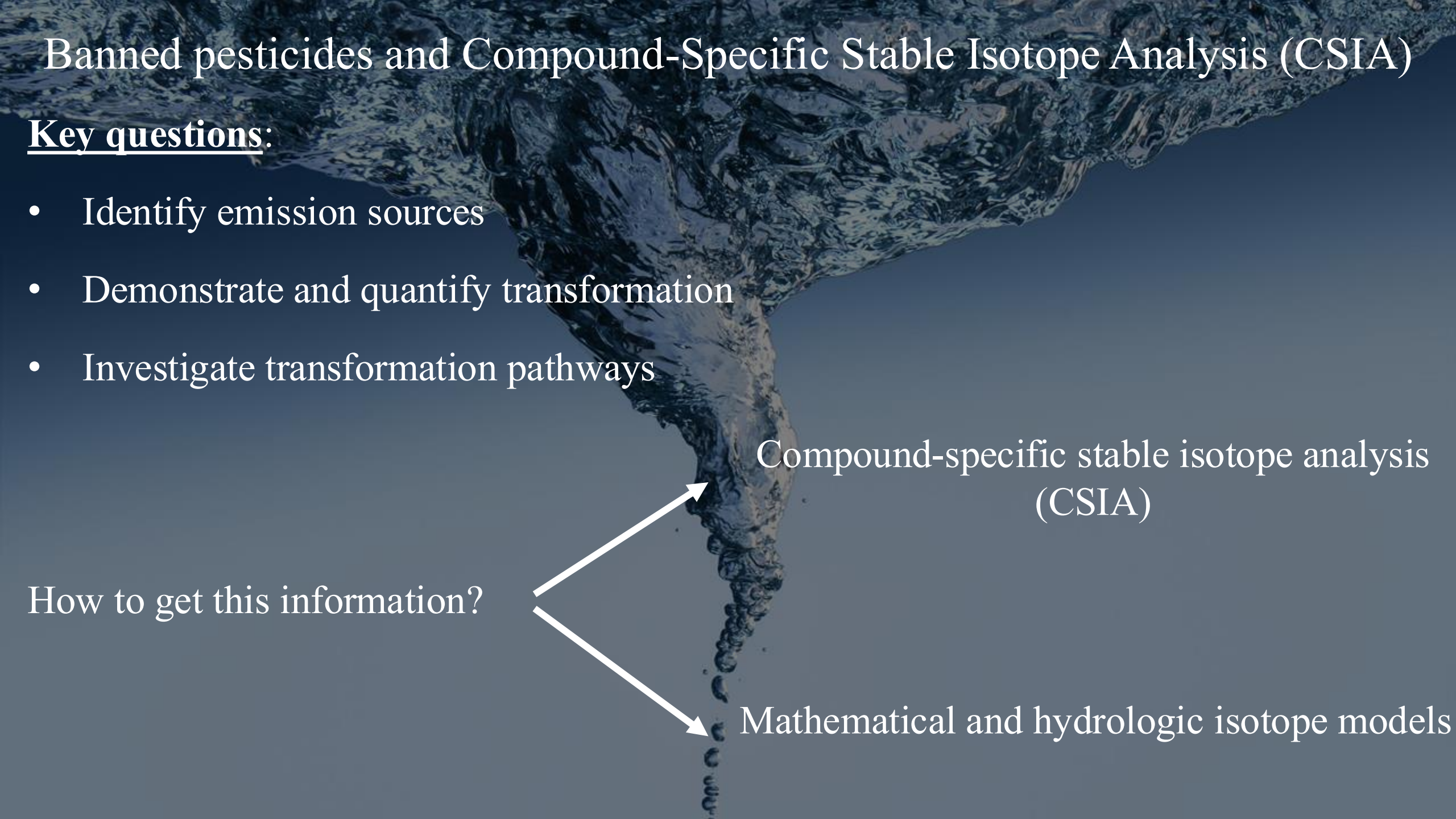
Key questions:

- Identify emission sources
- Demonstrate and quantify transformation
- Investigate transformation pathways

Compound-specific stable isotope analysis
(CSIA)

How to get this information?

Mathematical and hydrologic isotope models



Terminology: Isotope Ratio

Isotope Ratio: the ratio between the number of atoms of one stable isotope and the number of atoms of another stable isotope of the same element in the same sample.

$$R(^hE/^lE)_p = \frac{n(^hE)_p}{n(^lE)_p} = \frac{\text{number of atoms of the heavier isotope}}{\text{number of atoms of the lighter isotope}}$$



In one mole of C₆H₆, how many atoms of

¹²C

¹³C

If we wanted to quantify the isotope ratio between carbon-12 and carbon-13 in one mole of benzene, we would obtain:

$$R(^{13}\text{C}/^{12}\text{C})_{\text{C}_6\text{H}_6} = \frac{n(^{13}\text{C})_{\text{C}_6\text{H}_6}}{n(^{12}\text{C})_{\text{C}_6\text{H}_6}} = \frac{\text{total number of } ^{13}\text{C} \text{ atoms}}{\text{total number of } ^{12}\text{C} \text{ atoms}}$$

Terminology: δ notation

Because isotope ratios are very small numbers, the δ notation was introduced and is defined by the following equation:

$$\delta^h E_{s/Ref} = \left(\frac{R(^h E / ^l E)_{sample}}{R(^h E / ^l E)_{ref}} - 1 \right) \cdot 1000\text{‰}$$

R_{sample} = Isotopic ratio of the element of interest in the sample under investigation

$R_{reference}$ = Isotopic ratio of the element of interest defined by an internationally accepted reference scale.

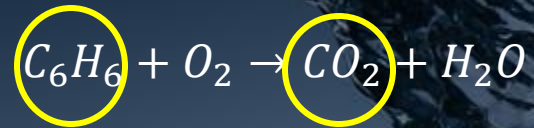
$$\delta^{13}C_{sample/VPDB} = \left(\frac{\left(\frac{\text{number of } ^{13}C \text{ atoms}}{\text{number of } ^{12}C \text{ atoms}} \right)_{sample}}{\left(\frac{\text{number of } ^{13}C \text{ atoms}}{\text{number of } ^{12}C \text{ atoms}} \right)_{VPDB}} - 1 \right) \cdot 1000\text{‰}$$

For carbon isotope ratios, the internationally accepted reference scale is VPDB (Vienna Pee Dee Belemnite), a marine carbonate material with a $^{13}C/^{12}C$ isotope ratio of 0.0111802. By convention, this reference ratio corresponds to a $\delta^{13}C$ value of 0‰ and therefore defines the zero point of the $\delta^{13}C$ scale.

Terminology: isotope fractionation factor

The isotopic ratio, R , and consequently the δ value, may vary as a result of:

chemical reaction



physical process



When a molecule undergoes a change in the isotopic ratio of an element, it is said to undergo isotopic fractionation, α .

$$\alpha(^i\text{E}/^j\text{E})_{P/R} = \frac{\frac{n(^i\text{E})_P}{n(^j\text{E})_P}}{\frac{n(^i\text{E})_R}{n(^j\text{E})_R}} = \frac{R(^h\text{E}/^l\text{E})_P}{R(^h\text{E}/^l\text{E})_R} = \frac{\delta^h(^h\text{E}/^l\text{E})_P + 1}{\delta^h(^h\text{E}/^l\text{E})_R + 1}$$

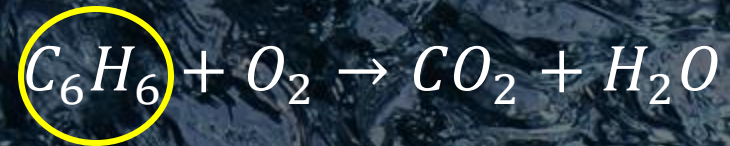
The fractionation factor, α , is often expressed in terms of ϵ , the enrichment factor, which is useful for describing the distribution of isotopes between substances

$$\epsilon^h E_{P/R} = \left(\alpha^h E_{\frac{P}{R}} - 1 \right) \cdot 1000\text{‰}$$

EXAMPLE

$$\delta^{13}\text{C}_{t=0} = -28.00\text{‰}$$

$$^{13}\text{R}_{t=0} = 0.0109225$$



Hypothetical values		
$R(^h\text{E}/^l\text{E})_R = \frac{n(^h\text{E})_R}{n(^l\text{E})_R}$	$\delta^h\text{E}_{R/\text{Ref}E} = \left(\frac{R(^h\text{E}/^l\text{E})_R}{R(^h\text{E}/^l\text{E})_{\text{ref}}} - 1 \right)$	$\Delta\delta = (\delta^{13}\text{C}_{t=t} - \delta^{13}\text{C}_{t=0})$
$^{13}\text{R}_{t=t1} = 0.0109786$	$\delta^{13}\text{C}_{t=t1} = -23.00\text{‰}$	> 0
$^{13}\text{R}_{t=t1} = 0.0108776$	$\delta^{13}\text{C}_{t=t1} = -32.00\text{‰}$	< 0

Reagent (C ₆ H ₆)		
$R(^h\text{E}/^l\text{E})_R = \frac{n(^h\text{E})_R}{n(^l\text{E})_R}$	$\delta^h\text{E}_{R/\text{Ref}E} = \left(\frac{R(^h\text{E}/^l\text{E})_R}{R(^h\text{E}/^l\text{E})_{\text{ref}}} - 1 \right)$	$\Delta\delta = (\delta^{13}\text{C}_{t=t} - \delta^{13}\text{C}_{t=0})$
↑ ¹³C	↑ ¹³C	↑ ¹³C
↑ ¹²C	↑ ¹²C	↑ ¹²C

Compound Specific Stable Isotope Analysis (CSIA)

Degradation of the ^{12}C -contaminant: $\longrightarrow \frac{d^{12}\text{C}}{dt} = -k_L \cdot [^{12}\text{C}]$

Degradation of the ^{13}C -contaminant: $\longrightarrow \frac{d^{13}\text{C}}{dt} = -k_H \cdot [^{13}\text{C}]$

Integration leads to the Rayleigh equation: $\longrightarrow R_t = R_0 \cdot f^{(\alpha-1)}$

$R_t = \frac{^{13}\text{C}}{^{12}\text{C}}$ = isotope ratio at a given time, t

$R_0 = \frac{^{13}\text{C}}{^{12}\text{C}}$ = isotope ratio at initial time, t_0

$f = \frac{C_t}{C_0}$ = remaining fraction of the compound being studied

$\alpha = \frac{k_H}{k_L}$ = (Kinetic) isotope fractionation factor

$(\alpha - 1) = \varepsilon$ = (Kinetic) isotope enrichment factor

Compound-Specific Stable Isotope Analysis (CSIA)

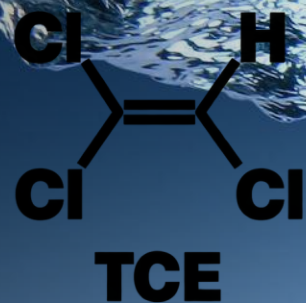
Changes in concentration and isotope ratios over time are related to each other? **➡ YES**

$$R_t = R_0 \cdot f^{(\alpha-1)} \quad \longrightarrow \quad \frac{R_t}{R_0} = \left(\frac{C_t}{C_0} \right)^{(\alpha-1)} \quad \longrightarrow \quad \left(\frac{\delta^{13}C_{\frac{sample}{STD},t} + 1}{\delta^{13}C_{\frac{sample}{STD},t_0} + 1} \right) = \left(\frac{C_t}{C_0} \right)^{(\alpha-1)}$$

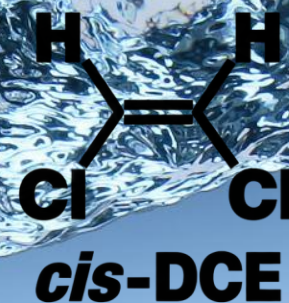
This mathematical relationship provides a powerful analytical tool because:

- During (bio)degradation, changes occur in both the concentration and the isotope ratio ($C_t \neq C_0$, $R_0 \neq R_t$, $\delta_0 \neq \delta_t$)
- During dilution (or concentration), only the concentration changes, while the isotope ratio remains constant ($C_t \neq C_0$, $R_0 = R_t$, $\delta_0 = \delta_t$)
- During physical processes (e.g., sorption and dispersion), the isotope ratio remains nearly constant, with any changes being within instrumental error. ($R_0 \approx R_t$, $\delta_0 \approx \delta_t$)

Compound-Specific Stable Isotope Analysis (CSIA)

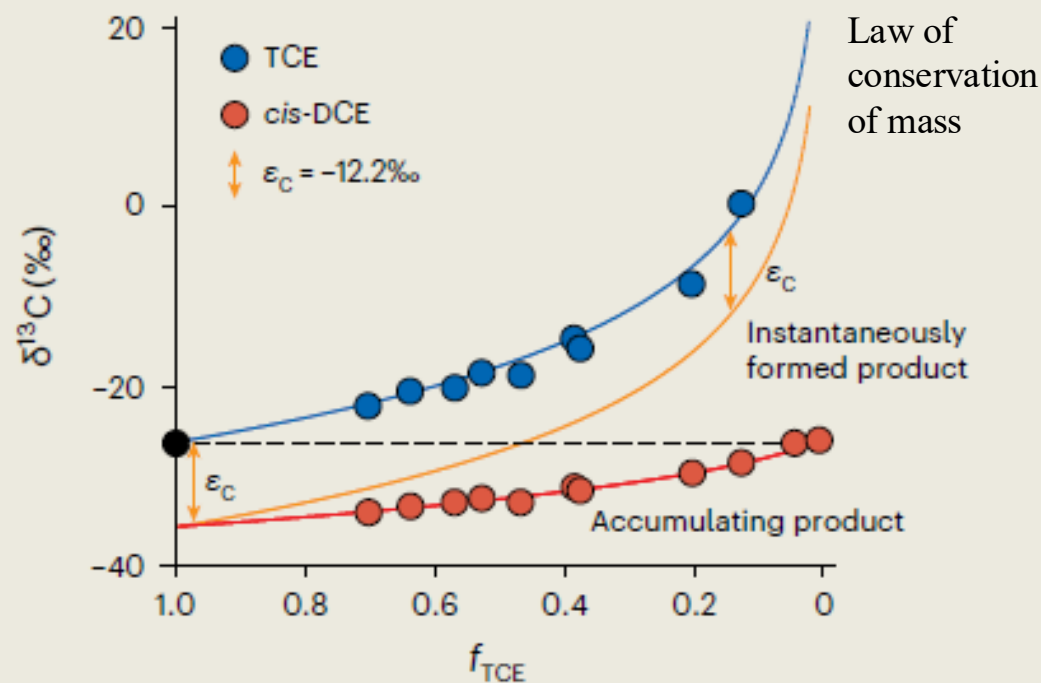


reductive dechlorination



CONDITIONS:
pH = 7
T = (15÷30) °C

by organohalide-respiring bacteria



$$\frac{\delta^{13}\text{C}_{\text{TCE},t} + 1}{\delta^{13}\text{C}_{\text{TCE},0} + 1} = \left(\frac{C_{\text{TCE},t}}{C_{\text{TCE},0}} \right)^{\epsilon_c}$$

$$\frac{\delta^{13}\text{C}_{\text{cis-DCE}} + 1}{\delta^{13}\text{C}_{\text{TCE},0} + 1} = \frac{1 - f^{(\epsilon_c + 1)}}{1 - f}$$

$$\delta^{13}\text{C}_{\text{prod,inst}} = \alpha(\delta^{13}\text{C}_{\text{TCE},t} + 1) - 1$$

$$\epsilon_c = \left(\frac{{}^{13}k}{{}^{12}k} - 1 \right) \times 1000\text{‰}$$

Conclusion: How can CSIA be applied in practice?

1. Characterize the contaminant

Measure concentration and isotope composition

2. Monitor over time

Track changes in concentration and δ -value

3. Identify the process

Concentration $\downarrow$ + δ unchanged $\rightarrow$ dilution / physical processes are likely

Concentration $\downarrow$ + δ changes $\rightarrow$ evidence of degradation / transformation

4. Quantify transformation

Use Rayleigh modelling to estimate the extent of degradation

5. Reconstruct contaminant fate

Combine CSIA + hydrological information to understand transport and transformation in the catchment

CSIA provides information that concentration measurements alone cannot provide.



THANK YOU

References

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GC-IRMS-MS: How does it work?



GC-IRMS-MS: How does it work?

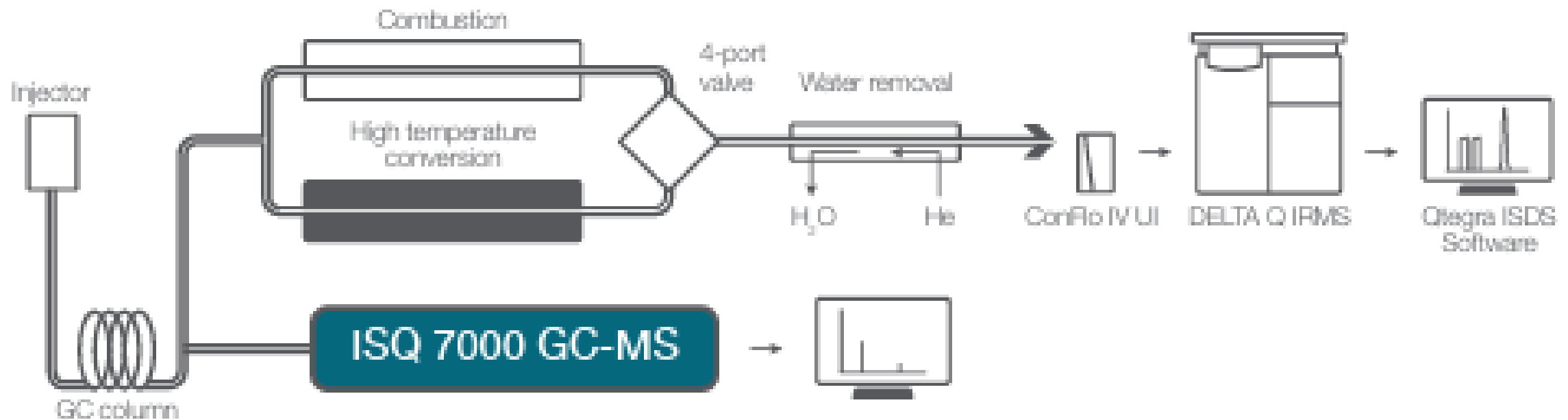


Figure 2. GC-MS-IRMS System workflow