

# Quantifying the contribution of three competing pathways to total degradation in groundwater by a triple-isotope analysis approach

Martin Thullner<sup>1</sup>, Florian Centler<sup>1</sup>, Thomas Hofstetter<sup>2</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany; <sup>2</sup>Eawag – Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

# Introduction

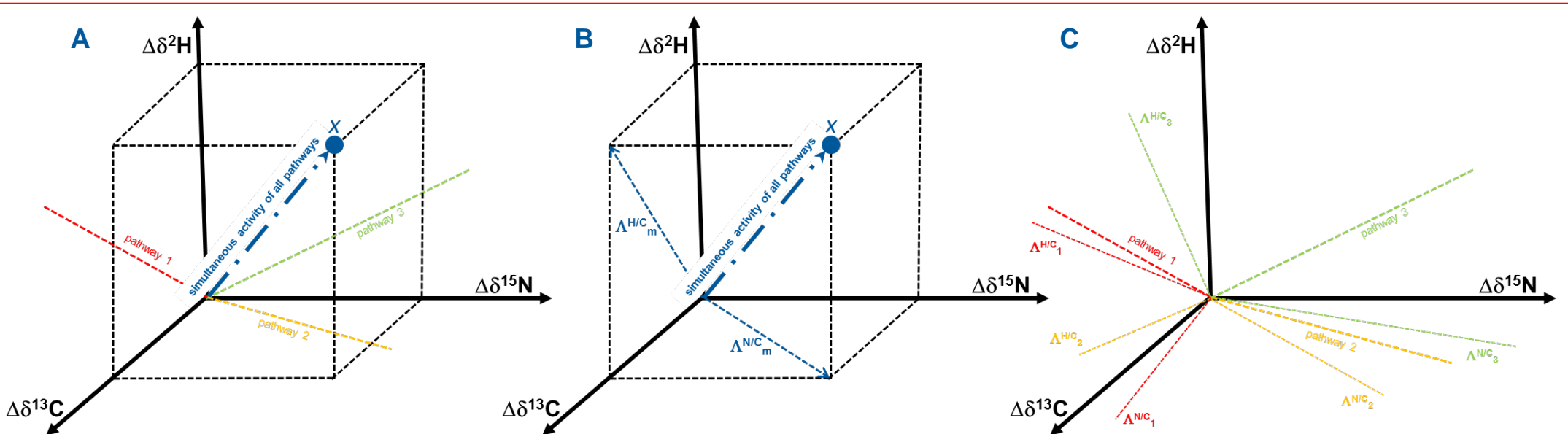
Multi-element compound-specific stable isotope analysis (CSIA) has been used for the determination of specific degradation pathways. The 'dual-isotope' or two-dimensional isotope' analysis also allows for an estimation of the contribution of two different pathways contributing both to the overall degradation [1].

Recent CSIA approaches also allow for investigating the simultaneous stable isotope fractionation effects for three different elements. Such triple-isotope analysis of a degradable compound has been used for a qualitative analysis of the contribution of three different degradation pathways, but up to now there is no theoretical concepts providing a quantitative estimate.

The aim of the present study is to overcome this shortage and to present such theoretical concept for the quantification of single pathway contribution to the overall biodegradation in systems with three parallel degradation pathways.

## Computational approach

Stable isotope data for each analyzed element of a compound (here H, C, N as examples) are given using the  $\delta$ -notation. Stable isotope fractionation during degradation leads to a shift of the  $\delta$ -values in the remaining compound (i.e.  $\Delta\delta^2\text{H}$ ,  $\Delta\delta^{13}\text{C}$ ,  $\Delta\delta^{15}\text{N}$ ). We consider three degradation pathways each contributing to the stable isotope fractionation of the compound. For one of the elements (here arbitrarily C) the stable isotope enrichment factors for each pathway ( $\varepsilon_{\text{C}_1}$ ,  $\varepsilon_{\text{C}_2}$ ,  $\varepsilon_{\text{C}_3}$ ) must be known. Also the ratios  $\Lambda^{\text{H/C}}_i = \varepsilon_{\text{H}}/\varepsilon_{\text{C}_i}$  and  $\Lambda^{\text{N/C}}_i = \varepsilon_{\text{N}}/\varepsilon_{\text{C}_i}$  between the enrichment factors for the other two elements must be known for each pathway  $i$ .



Schematic representation of the triple isotope analysis. For a given sample of the degraded compound the simultaneous activity of all pathways defines a specific point in the three-dimensional  $\Delta\delta^2\text{H} - \Delta\delta^{13}\text{C} - \Delta\delta^{15}\text{N}$  - plot (panel A). If degradation would have been determined by a single pathway only data point would plot along a trajectory specific for the pathway and any deviation from such trajectories indicates the presence of other pathway(s). Pairwise analysis of measured  $\delta$ -values of two elements in a dual isotope analysis provides slopes  $\Delta^{H/C}_m$  and  $\Delta^{N/C}_m$  (panel B). For the single pathways the corresponding slopes (panel C) are given by the ratios of the enrichment factors.

The contribution of each of the three pathway is given by the ratio  $F_i$  between the unknown rates  $r_i$  or each pathway  $i$ . Assuming that this ratio remains constant during degradation and expanding the approach of [1] its value is given as:

$$F_i = \frac{r_i}{r_1 + r_2 + r_3} = \frac{X_i}{X_1 + X_2 + X_3} \quad \text{with} \quad X_1 = \varepsilon^C_2 \varepsilon^C_3 \left[ \left( \Lambda_m^{N/C} - \Lambda_2^{N/C} \right) \left( \Lambda_m^{H/C} - \Lambda_3^{H/C} \right) - \left( \Lambda_m^{H/C} - \Lambda_2^{H/C} \right) \left( \Lambda_m^{N/C} - \Lambda_3^{N/C} \right) \right],$$

$$X_2 = \varepsilon^{\mathcal{C}_1} \varepsilon^{\mathcal{C}_3} \left[ \left( \Lambda_m^{H/c} - \Lambda_1^{H/c} \right) \left( \Lambda_m^{N/c} - \Lambda_3^{N/c} \right) - \left( \Lambda_m^{N/c} - \Lambda_1^{N/c} \right) \left( \Lambda_m^{H/c} - \Lambda_3^{H/c} \right) \right] \text{ and } X_3 = \varepsilon^{\mathcal{C}_1} \varepsilon^{\mathcal{C}_2} \left[ \left( \Lambda_m^{N/c} - \Lambda_1^{N/c} \right) \left( \Lambda_m^{H/c} - \Lambda_2^{H/c} \right) - \left( \Lambda_m^{H/c} - \Lambda_1^{H/c} \right) \left( \Lambda_m^{N/c} - \Lambda_2^{N/c} \right) \right]$$

which allows determining the pathway contribution based on stable isotope data only.

## Application to measured data

Data are taken from [2] describing the degradation of 2,4-DNT three pathways (1: dioxygenation, 2: reduction, 3: oxygenation). Measured data exhibit  $\Delta^{H/C}_m=8.8$  and  $\Delta^{N/C}_m=2.5$ . Using pathway specific data from independent measurements the contribution  $F_i$  of each pathway can be obtained:

Pathway	1	2	3
$\varepsilon_C$	-0.5‰	-0.6‰	-2.2‰
$\Lambda^{H/C}$	7	13	58
$\Lambda^{N/C}$	1.5	14	1.1
<b>F</b>	<b>92%</b>	<b>7%</b>	<b>1%</b>

This is in agreement to values from [2] using a different estimation approach.

## Conclusions

- The presented approach provides quantitative estimates for the contribution of three different pathways to the overall degradation using solely measured stable isotope signatures and pathway specific stable isotope fractionation information.
- The approach can be applied to any system with three different degradation pathways causing a stable isotope fractionation of three elements as long as the abundance of the heavy isotope is low (i.e.  $R = C^{\text{heavy}}/C^{\text{light}} \ll 1$ ).
- The approach could be successfully applied to the measured data but further verification/testing would be desirable.

## References

- [1] Centler, F., Hesse, F., and Thullner, M. (2013) *Journal of Contaminant Hydrology*, 152, 97-116.
- [2] Wijker, R. S., Bolotin, J., Nishino, S. F., Spain, J. C., and Hofstetter, T. B. (2013) *Environmental Science & Technology*, 47, 6872-6883.