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1 Quantification of Lambda (Λ) in multi-elemental compound- 2 specific isotope analysis

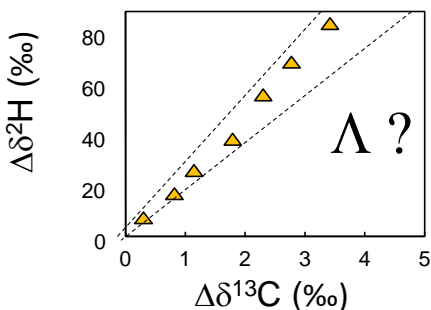
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11 Highlights

12 The parameter Λ represents dual element stable isotope data

13 Two conventions for quantifying Λ give different Λ values

14 Linear regressions of delta values in a dual element plot overestimate Λ

15 We show that only the ln-transformed isotope ratios should be fitted

16

17

18 ABSTRACT

19 In multi-elemental compound-specific isotope analysis the lambda (Λ) value expresses the
20 isotope shift of one element versus the isotope shift of a second element. In dual-isotope plots,
21 the slope of the regression lines typical reveals the footprint of the underlying isotope effects
22 allowing to distinguish degradation pathways of an organic contaminant molecule in the
23 environment. While different conventions and fitting procedures are used in the literature to
24 determine Λ , it remains unclear how they affect the magnitude of Λ . Here we generate synthetic
25 data for benzene $\delta^2\text{H}$ and $\delta^{13}\text{C}$ with two enrichment factors ε_H and ε_C using the Rayleigh equation
26 to examine how different conventions and linear fitting procedures yield distinct Λ . Fitting an
27 error-free data set in a graph plotting the $\delta^2\text{H}$ versus $\delta^{13}\text{C}$ overestimates Λ by $0.225\% \cdot \varepsilon_H/\varepsilon_C$,
28 meaning that if $\varepsilon_H/\varepsilon_C$ is larger than 22, Λ is overestimated by more than 5%. The correct fitting
29 of Λ requires a natural logarithmic transformation of $\delta^2\text{H}$ versus $\delta^{13}\text{C}$ data. Using this
30 transformation, the ordinary linear regression (OLR), the reduced major-axis (RMA) and the
31 York methods find the correct Λ , even for large $\varepsilon_H/\varepsilon_C$. Fitting a dataset with synthetic data with
32 typical random errors let to the same conclusion and positioned the suitability of each regression
33 method. We conclude that fitting of non-transformed δ values should be discontinued. The
34 validity of most previous Λ values is not compromised, although previously obtained Λ values
35 for large $\varepsilon_H/\varepsilon_C$ could be corrected using our error estimation to improve comparison.

36 Key Words

37 Stable isotopes, pollution, assessment, bioremediation

38 1. Introduction

39 Multi-elemental Compound-Specific Isotope Analysis (ME-CSIA) is increasingly used to assess
40 the fate of pollutants such as hydrocarbons (Vogt et al., 2016), chlorinated solvents solvents
41 (Palau et al., 2014, Audi-Miro et al., 2015, Palau et al., 2016), nitrates (Xue et al., 2009),
42 perchlorates (Sturchio et al., 2012) and pesticides (Ponsin et al., 2019, Melsbach et al., 2020) in
43 the environment. The slope of the dual-isotope plot (Lambda, Λ) reflects changes of the isotope
44 ratios of each element, which can be specific to a reaction mechanism, and thus inform about
45 transformation processes in the laboratory or in the field. (Vogt et al., 2016, Elsner, 2010)
46 Several studies (Masbou et al., 2018, Huntscha et al., 2014, Lian et al., 2019, Bouchard et al.,
47 2018, Vogt et al., 2016, Elsner, 2010, Ojeda et al., 2019) refer to Λ using the simple definition in
48 eq. 1, which is written here as an example for hydrogen vs carbon δ values (eq. 1).

$$49 \Lambda = \frac{\Delta\delta^{2H}}{\Delta\delta^{13C}} \approx \frac{\epsilon_H}{\epsilon_C} \quad \text{eq. 1}$$

50 where $\Delta\delta$ is the change of isotope ratios from initial values, and ϵ are the enrichment factors for
51 hydrogen and carbon. The Lambda (Λ) is an important parameter in ME-CSIA. It is a practical
52 and unitless number which characterizes a specific process. It can be determined either by simply
53 using the two enrichment factors and the right-hand side of equation 1 on one hand, or from
54 regression analysis in a dual-isotope plots with isotope data of one element versus data of
55 another element in the same compound (Figure 1). Lambda values were obtained in many studies
56 (Ojeda et al., 2019, Palau et al., 2017, Rosell et al., 2007, Rodriguez-Fernandez et al., 2018,
57 Rodriguez-Fernandez et al., 2018, Dogan-Subasi et al., 2017, Cretnik et al., 2013, Audi-Miro et
58 al., 2013, Palau et al., 2014, Lian et al., 2019, Badin et al., 2016, Mogusu et al., 2015, Ponsin et

59 al., 2019, McKelvie et al., 2009, Pati et al., 2012) from the regression analyses in dual-isotope
60 plots (i.e., ratios of one isotope as a function of another isotope as delta values; Figure 1A).

61 Another mathematical notation for Λ has been described in detail in (Wijker et al., 2013) (eq. 2),
62 noted here for hydrogen and carbon isotopes:

$$63 \quad \Lambda = \frac{\ln\left[\frac{(\delta^{2H}/1000 + 1)}{(\delta^{2H_0}/1000 + 1)}\right]}{\ln\left[\frac{(\delta^{13C}/1000 + 1)}{(\delta^{13C_0}/1000 + 1)}\right]} \approx \frac{\varepsilon_H}{\varepsilon_C} \quad \text{eq. 2}$$

64 Figure 1B shows an example of a dual-isotope plot to determine Λ using eq. 2, named below the
65 ln-transformed δ data. This way of obtaining Λ was used e.g. in (Schilling et al., 2019 a+b).

66 Apart from those two different conventions for plotting isotope data, different methods of linear
67 regression were proposed to obtain Λ . These include the ordinary linear regression (OLR), the
68 reduced major axis regression (RMA), and the York linear regression, which have been
69 compared recently (Ojeda et al., 2019).

70 The objective of this short comment is to compare the two conventions (i.e., A, with eq. 1 and B,
71 with 2) to determine Λ values and the associated uncertainty from a dual-isotope plot. Two
72 synthetic datasets were generated, one without random error, and a second one with random
73 errors mimicking measurement uncertainties. Each dataset was fitted with the ordinary linear
74 regression (OLR), the reduced major-axis (RMA) and the York regression methods and results
75 were compared.

76 **2. Methods**

77 The Rayleigh equation (eq. 3) (Aelion et al., 2010) was used to generate 10 synthetic exact data
78 points for each element (i.e., C and H). We used isotope enrichment factors for carbon and

79 hydrogen corresponding to methanogenic degradation of benzene: $\epsilon_C = -2.0$ and $\epsilon_H = -59.5$ ‰.
80 (Mancini et al., 2003) The remaining fraction (f) of benzene was varied from 1 to 0.1 in steps of
81 0.1 (see data set in the supplementary data).

$$82 \quad \frac{R}{R_0} = f^{(\alpha-1)} \quad \text{eq. 3}$$

83 Where R is the isotope ratio, R_0 is the initial isotope ratio (chosen as the R of international
84 standard R_{std}), f is the fraction of compound remaining (C/C_0), and α is the isotope fractionation
85 factor (equal to $\epsilon/1000 + 1$). The resulting isotope ratios were expressed as δ values [$\delta = (R/R_{\text{std}} -$
86 $1) * 1000$; $R_{\text{std,H}} = 1.5575\text{E-}4$; $R_{\text{std,C}} = 0.011237$] and plotted in Figure 1A ($\Delta\delta^2\text{H}$ vs $\Delta\delta^{13}\text{C}$, eq. 1)
87 and 1B (ln-transformed data, eq. 2). The resulting slopes should reflect the ratio of original
88 isotopic enrichment values, $-59.50/-2.00$, thus $\Lambda = 29.75$.

89 A second dataset was generated using the same enrichment factors but introducing random errors
90 in the calculated δ values (see Table S1 in supplementary data). The δ values of this set had a
91 random error of up to ± 0.5 ‰ for carbon and up to ± 5.0 ‰ for hydrogen, which corresponds to
92 the typical total analytical uncertainties.

93 Finally, 25 more datasets (data not shown) were generated in the same manner as dataset 1
94 without random error, keeping $\epsilon_C = -2.0$ ‰ and varying ϵ_H over ϵ_H / ϵ_C ratios from 2 to 50. Each
95 of these data sets was fitted with OLR, and the overestimation of fit A over fit B was quantified
96 and plotted in Figure 2 as a function of ϵ_H / ϵ_C .

97 The datasets were generated with Excel (Microsoft), Vs. 2011), and linear regressions (OLR,
98 RMA and York) were calculated with a script adapted from Ojeda et al. (2019) and were not
99 forced through the origin.

100 3. Results

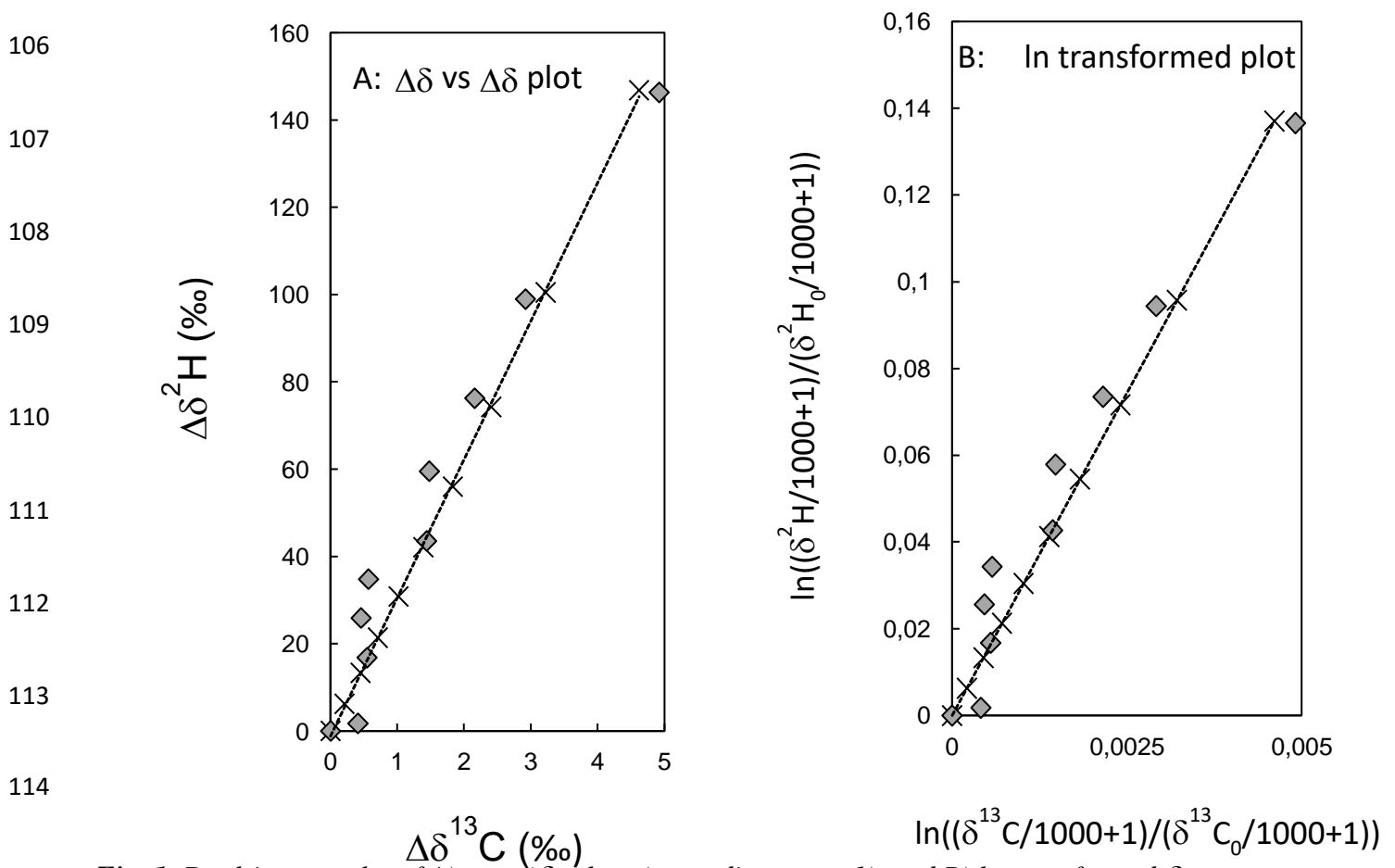
101 The dataset 1 with the raw $\Delta\delta$ values (eq. 1) does not plot on a perfect straight line (Figure 1A).

102 The slope becomes steeper with increasing δ values (smaller f). An OLR gives a mean Λ of

103 31.70 ± 0.21 ($R^2 > 0.99$), which overestimates the true Λ of 29.75 by 6.6 %. In contrast, the

104 dataset 1 with ln-transformed δ values (eq. 2) plots perfectly on a straight line with a slope of

105 29.74 ± 0.02 with an R^2 of 1.0000 (Figure 1B), which matches the true Λ .



115 **Fig. 1.** Dual-isotope plot of A) raw $\Delta\delta$ values (according to eq. 1), and B) ln-transformed δ

116 values (according to eq. 2). Crosses correspond to exact datapoints (dataset 1) and grey

117 diamonds are datapoints with random error (dataset 2).

118

119 **Table 1:** Comparison of Λ calculated with the raw $\Delta\delta$ values (convention A, eq. 1) and the \ln -transformed δ values (convention B, eq.
 120 2) using the OLR, RMA and York methods, for the exact data points (dataset 1) and data generated with a random error (dataset 2).

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127

<i>Exact data points (dataset 1)</i>							<i>Random error (dataset 2)</i>					
<i>$\Delta\delta$ vs $\Delta\delta$</i>				<i>\ln-transformed</i>			<i>$\Delta\delta$ vs $\Delta\delta$</i>			<i>\ln-transformed</i>		
	<i>Λ</i>	<i>SE</i>	<i>R^2</i>	<i>Λ</i>	<i>SE</i>	<i>R^2</i>	<i>Λ</i>	<i>SE</i>	<i>R^2</i>	<i>Λ</i>	<i>SE</i>	<i>R^2</i>
<i>OLR</i>	31.70	0.21	>0.99	29.74	0.02	1.00	30.08	2.13	0.96	28.15	2.16	0.95
<i>RMA</i>	31.71	0.19	>0.99	29.74	0.02	1.00	30.67	1.90	0.98	28.80	1.93	0.98
<i>York</i>	31.71	3.77	>0.99	29.74	3.56	1.00	31.17	3.68	0.96	29.33	3.50	0.95

122 *SE: Standard error of Λ*

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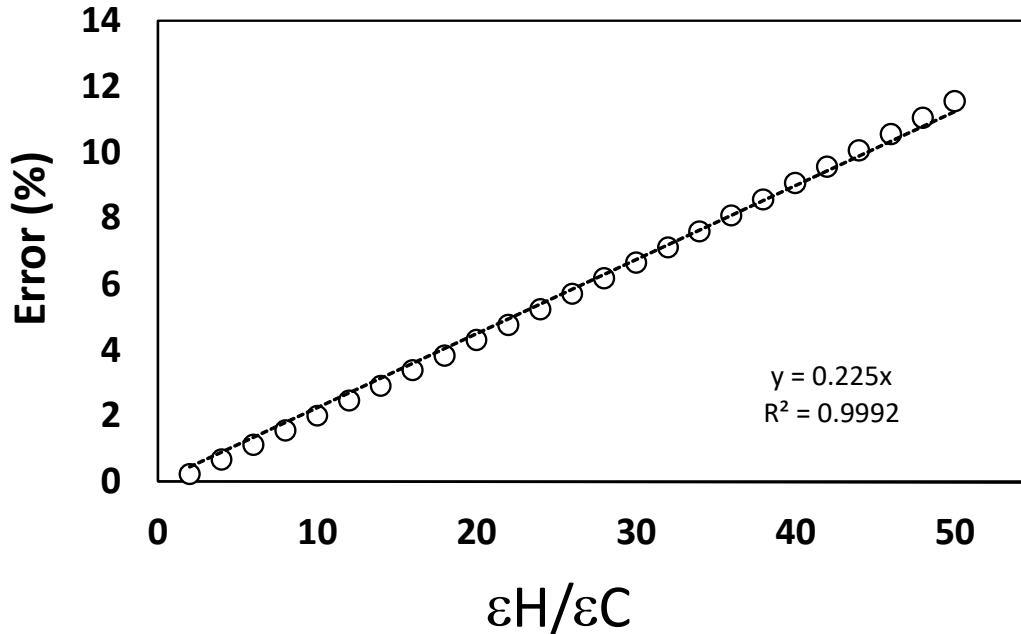
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129 The overestimation of Λ calculated with convention A compared to convention B was quantified
 130 as a function of $\varepsilon_H/\varepsilon_C$ ranging from 2 to 50 (Fig. 2; OLR method)).



131

132 **Fig. 2** Overestimation of Λ (%) as a function of $\varepsilon_H/\varepsilon_C$ (symbols) when convention A (eq. 1) is
 133 used. The straight dotted line is the mean error increase of 0.225% per $\varepsilon_H/\varepsilon_C$.

134 Figure 2 shows that the error in a graph plotting the δ values like in Fig. 1.A overestimates Λ by
 135 11.5 % when $\varepsilon_H / \varepsilon_C$ reaches 50. The increase of the error is almost linear with a slope of 0.225%
 136 per $\varepsilon_H / \varepsilon_C$.

137 4. Discussion

138 The use of the exact (error-free) synthetic dataset to compare conventions A (eq. 1) and B (eq. 2)
 139 emphasized that Λ calculated with convention A is linearly overestimated (eq.1). The difference

140 of Λ obtained with convention A and B has a pure mathematical cause (Wijker et al., 2013):
141 equation 1 is derived from eq. 2 by a Taylor series expansion which is only approximate.

142 Höhener and Atteia (Höhener and Atteia, 2014) derived mathematically the dependence of the
143 slope Λ on the remaining, non-degraded fraction f in a dual-isotope plot (eq. 4) based on the
144 theory of Rayleigh distillation.

$$145 \quad \Lambda = \frac{\Delta\delta^{2H}}{\Delta\delta^{13C}} = \frac{f^{\frac{\epsilon_H}{1000}-1}}{f^{\frac{\epsilon_C}{1000}-1}} \quad \text{eq. 4}$$

146 Equation 4 (eq. 16 in (Höhener and Atteia, 2014)) shows that Λ is increasing with decreasing f ,
147 as observed in Figure 1A. Thus, for f close to one, Λ is 29.75, while for $f = 0.1$, Λ is 31.80.

148 All three regression methods tested for convention A with dataset 1 gave a similar Λ of 31.7,
149 although their standard errors (SE) differed (Table 1). OLR and RMA methods gave a narrow SE
150 (0.21 and 0.19, respectively), leading us to the wrong conclusion that Λ is > 31 . Regression with
151 the York method gave a larger SE ($\Lambda = 31.71 \pm 3.68$, Tab. 1), which represents a correct but
152 inaccurate description of the true Λ of 29.75. For convention B and dataset 1, all three regression
153 methods find the true Λ , although only the OLR and RMA method yielded accurate Λ within
154 narrow error limits.

155 Measured isotope ratios are always affected by random errors from measurements, which were
156 accounted for in dataset 2 to calculate Λ (Table 1). All three methods predicted $\Lambda > 31$ using
157 convention A, and Λ was associated with large SE, ranging from 1.90 to 3.68. Using convention
158 B, Λ ranged from 28.15 to 29.33, with SE ranging from 1.9 (RMA) to 3.5 (York). For dataset 2,
159 RMA was the best fitting method, yielding the narrower SE, while both OLR and York gave

160 accurate predictions also with higher error. All regressions match thus the true value of 29.75
161 within their error limits.

162 To sum up, the error-free data in a dual-isotope plot with $\Delta\delta$ vs $\Delta\delta$ values do not lie on a straight
163 line and thus should not be fitted with any linear regression. The slope in a $\Delta\delta$ vs $\Delta\delta$ plot is per
164 definition a function of the progress of reaction f (eq. 4). A non-linear curve is obtained,
165 especially when the orders of magnitude of the enrichment factors differ. Linear regressions in
166 such plots yield Λ that overestimate the true Λ and should be discontinued. The correct
167 convention to linearize data is provided in eq. 2 and should be applied as in Figure 1B to obtain
168 accurate Λ . OLR and RMA regression methods yield narrower error estimates, whereas the York
169 method finds the true Λ within a larger error margin. The validity of most previously obtained Λ
170 values with convention A might not be compromised given the total uncertainty of the
171 experimental and analytical methods. However, in a few cases with large $\varepsilon_H/\varepsilon_C$ ratios, corrections
172 might be applied in order to compare optimally all Λ values. The simple procedure to follow
173 consists in using Fig. 2 of our manuscript, selecting the appropriate ratio of epsilons, reporting
174 the corresponding error percentage (which is the percentage of overestimation) to lower Λ by this
175 percentage. Worthy of note, if experimental data still plotting nonlinearly on a ln-transformed
176 plot with eq. 2, as e.g. in (Dorer et al., 2014), another process may be involved, including a very
177 strong hydrogen fractionation (tunneling), concentration-dependent fractionation and/or
178 instrumental non-linearity. In these specific cases, Λ cannot be expressed as a constant number.

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182 Supplementary data

183 Table of synthetic datasets used in this work.

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303 **SUPPLEMENTARY DATA**304 **Quantification of Lambda (Λ) in multi-elemental compound-specific isotope**
305 **analysis**306 **¹Patrick Höhener* and ²Gwenaël Imfeld**307 ¹Aix Marseille University – CNRS, UMR 7376, Laboratory of Environmental Chemistry, Marseille,
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311 7517 CNRS/EOST, 1 Rue Blessig, 67084, Strasbourg Cedex, France312 **Contents:**313 **Table S1: Datasets used in this work**314 *Table S1: Synthetic data shown in Figure 1 and used for fitting.*

Remaining fraction f	Exact data points (dataset 1)		Random error (dataset 2)	
	$\delta^{13}\text{C}$ ‰	$\delta^2\text{H}$ ‰	$\delta^{13}\text{C}$ ‰	$\delta^2\text{H}$ ‰
1	0	0	0	0
0.9	0.21	6.29	0.41	1.79
0.8	0.45	13.37	0.55	16.87
0.7	0.71	21.45	0.46	25.95
0.6	1.02	30.86	0.57	34.86
0.5	1.39	42.10	1.44	43.6
0.4	1.83	56.03	1.48	59.53
0.3	2.41	74.26	2.16	76.26
0.2	3.22	100.50	2.92	99.0
0.1	4.62	146.83	4.92	146.33

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