

1 **Isotope-Ratio Infrared Spectroscopy: a reliable tool for the investigation**
 2 **of plant-water sources?**

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26 **SUMMARY**

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• Stable isotopes are extensively used as tracers for the study of plant-water sources. Isotope-ratio infrared spectroscopy (IRIS) offers a cheaper alternative to isotope-ratio mass spectroscopy (IRMS), but its use in plant and soil water is limited by the spectral interference caused by organic contaminants. Here, we examine two approaches to cope with contaminated samples in IRIS: on-line oxidation of organic compounds (MCM) and post-processing correction.

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• We assessed these methods compared to IRMS across 136 samples of xylem and soil water and a set of ethanol- and methanol-water mixtures.

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• A post-processing correction improved significantly IRIS accuracy in both natural samples and alcohol dilutions, being effective with concentrations up to 8% of ethanol and 0.4% of methanol. MCM outperformed the post-processing correction in removing methanol interference, but was not effective for high concentrations of ethanol.

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• By using both approaches IRIS can overcome with reasonable accuracy the analytical uncertainties associated to most organic contaminants found in soil and xylem water. We recommend the post-processing correction as the first choice for the analysis of samples of unknown contamination. Nevertheless, MCM can be more effective for samples containing contaminants responsible of strong spectral interferences from small concentrations, such as methanol.

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Keywords:

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CRDS, ecohydrology, $\delta^{18}\text{O}$, $\delta^2\text{H}$, IRIS, IRMS, soil, xylem

50 **INTRODUCTION**

51 The stable isotope composition of oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$) in xylem water is
52 widely used as a tracer for the study of plants and fungi water uptake and redistribution
53 (Ehleringer & Dawson, 1992; Dawson, 1996; Warren *et al.*, 2008; Lilleskov *et al.*,
54 2009; Dawson & Simonin, 2011; Moreno-Gutiérrez *et al.*, 2012; Prieto *et al.*, 2012;
55 Palacio *et al.*, 2014a; Treydte *et al.*, 2014). Recently, the widespread use of isotope-ratio
56 mass spectrometry (IRMS) technology for measuring water isotopes has been
57 challenged by the development of isotope-ratio infrared spectroscopy (IRIS). IRIS
58 methods provide isotopic compositions of water samples by spectroscopy, taking
59 advantage of the different absorption spectra of water isotopologues in the gaseous
60 phase (Lis *et al.*, 2008; Gupta *et al.*, 2009). This allows the simultaneous measurement
61 of $^1\text{H}_2^{16}\text{O}$, $^1\text{H}_2^{18}\text{O}$, and $^1\text{H}^2\text{H}^{16}\text{O}$ with an accuracy comparable to IRMS, at least when
62 analysing pure water (Lis *et al.*, 2008; Brand *et al.*, 2009; Gupta *et al.*, 2009; West *et al.*,
63 2010, 2011). IRIS, unlike IRMS, does not need the prior chemical equilibration or
64 conversion into elemental constituents that often limits precision (Brand *et al.*, 2009;
65 Schultz *et al.*, 2011; Schmidt *et al.*, 2012). IRIS also offers other advantages such as
66 lower cost, easier installation and maintenance, and higher portability (Brand *et al.*,
67 2009; Gupta *et al.*, 2009; Berman *et al.*, 2009; West *et al.*, 2010; Johnson *et al.*, 2011;
68 Schmidt *et al.*, 2012).

69 However, some organic contaminants significantly interfere with the water-
70 isotope spectrum in IRIS analyses (Brand *et al.*, 2009; West *et al.*, 2010, 2011).
71 Organics are broadly found together with water in plant and soil samples, and cryogenic
72 distillation, which is the most common method for extracting water from plant and soil
73 matrices, frequently co-distils them. The magnitude of the error caused by organic
74 interference is not only proportional to the amount of contaminant, but also depends on
75 its spectral properties; for some compounds, the associated analytical errors may
76 become unacceptable starting at very small concentrations (e.g. <0.1% for methanol
77 (Brand *et al.*, 2009)). In contrast, the magnitude of the errors associated with
78 contaminants in IRMS depends on the mass-balance contribution of the contaminant to
79 the pool of H and O atoms in the sample. Hence, substantial errors can only be expected
80 for IRMS if the concentration of the contaminant is high and/or the isotopic
81 composition of the organic compound differs strongly from that of water (Brand *et al.*,
82 2009; West *et al.*, 2010).

83 IRIS manufacturers have developed software applications that can identify and
84 flag potentially contaminated samples (e.g. Spectral Contamination Identifier, Los
85 Gatos Research, Inc., Mountain View, CA; ChemCorrect™, Picarro Inc., Santa Clara,
86 CA). In the case of Picarro's, firstly ChemCorrect™ compares the measured spectral
87 profile of the sample with that of small molecules such as methane and methanol
88 contained in its library. If the features match, the compound concentration is calculated.
89 Afterwards, the software uses a set of quantitative spectral indicators (mainly spectral
90 baseline and slope) to generate information of larger organics, such as ethanol and other
91 alcohols, included in a 'C₂₊ alcohols' pool (Picarro, 2010; Richman *et al.*, 2010). Thus, a
92 set of organic-corrected spectra is currently available for post-processing correction in
93 the raw data files of L2110-*i* and L2120-*i* models. Later Picarro models (L2130-*i* and
94 L2140-*i* analysers) do not include this information in the raw data files, but a new post-
95 fit correction for these models is expected to be released in the future (Picarro
96 development team, personal communication). The protocol for deriving corrected
97 isotopic values from the spectral data is available for registered users in the Picarro
98 forum. However, it has not been extensively validated due to limited accessibility and,
99 therefore, not widely used to date.

100 More recently, Picarro Inc. has developed the Micro-Combustion Module™
101 (MCM) to remove organic compounds interfering with pure water; the MCM uses high-
102 temperature oxidation to eliminate problematic contaminants in the water sample
103 (Picarro, 2012; Saad *et al.*, 2013). Briefly, once a sample is evaporated the entire
104 gaseous phase is swept in a carrier gas across the heated metal catalyst in which
105 oxidation efficiently converts the organics into minute quantities of CO₂ and nascent
106 water. This procedure is expected to eliminate most common alcohols and other plant
107 contaminants of low molecular weight, including multicomponent mixtures of alcohols
108 and terpenes, and green-leaf volatiles. Its optimal efficacy is claimed to be achieved for
109 samples containing total organics at concentrations lower than 0.5%, with a complete
110 elimination for higher concentrations not entirely guaranteed. However, the
111 effectiveness of the MCM pre-treatment and post-processing corrections in soil and
112 xylem samples still requires full testing.

113 We present here the first evaluation of the performance of MCM using an array
114 of soil and xylem samples from a wide range of sites and species; these results are
115 compared with standard IRIS analyses without the MCM installed. We also present the
116 first validation of a post-processing method, based on ChemCorrect™ post-fit spectral

117 information, to reduce the effects of organic contamination on water isotopic analysis.
118 Both MCM and post-processed values are validated in field-collected samples against
119 IRMS, and further tested by using a set of standard dilutions of two representative
120 contaminants (methanol, MeOH, and ethanol, EtOH).
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122 MATERIALS AND METHODS

123

124 **Sample collection and water extraction**

125 We tested 136 samples from 26 species (xylem samples) and 8 sites (soil samples). The
126 samples were collected from a range of Mediterranean-type ecosystems in Spain and the
127 USA (Table 1) following the same standard procedure (Moisture Isotopes in the
128 Biosphere and Atmosphere -MIBA- protocol from International Atomic Energy Agency
129 -IAEA-, available at http://www.naweb.iaea.org/napc/ih/IHS_resources_miba.html).
130 The species belonged to 13 families, which were subsequently used as main taxonomic
131 units. Sunlit twigs were harvested near midday, bark and phloem were removed, and the
132 xylem was immediately sealed in glass vials (air-tight tubes, Duran GL-18). Soil
133 samples from different depths were simultaneously collected and were also rapidly
134 sealed in glass vials. The samples were placed on dry ice in the field and kept frozen
135 until processing.

136 The extraction of water from the soil and xylem samples was performed by
137 cryogenic vacuum distillation (Dawson & Ehleringer, 1993). Samples from the Iberian
138 Peninsula were processed at the Dept. of Crop and Forest Sciences, Universitat de
139 Lleida (Spain). The extraction system consisted of 10 sample tubes connected with
140 Ultra-Torr™ fittings (Swagelok Company, Solon, Ohio, USA) to 10 U-shaped
141 collection tubes specifically designed for this system. The sample tubes were
142 submerged in mineral oil at a constant temperature (110-120°C) to evaporate water and
143 the U-tubes were cooled with liquid nitrogen to condense the water vapour. The
144 extraction system was connected to a vacuum pump (model RV3; Edwards, Bolton,
145 UK) to guarantee the flow of water vapour from the sample tubes to the collection tubes
146 and to prevent contamination with atmospheric water vapour. The entire system
147 maintained constant vacuum pressures of *ca.* 10⁻² mbar. Distillation of sample collected
148 in the USA was conducted at the Center for Stable Isotope Biogeochemistry at the
149 University of California, Berkeley, CA, USA, using the same procedure but with a
150 slightly different design (Goldsmith *et al.*, 2012).

151

152 **Ethanol- and methanol-water mixtures**

153 To determine the influence that organic contaminants may have on the analysis of
154 isotope ratios of water using either IRIS or IRMS, we prepared a set of mixtures with
155 different concentrations of two organic compounds, EtOH and MeOH, representative of

156 broadband (baseline) and narrowband spectral interference, respectively (Schultz *et al.*,
 157 2011; Leen *et al.*, 2012). The mixtures were used as known “reference” samples by
 158 mixing EtOH or MeOH with water of known isotopic composition ($\delta^{18}\text{O} = -9.48\text{‰}$, $\delta^2\text{H}$
 159 $= -65.05\text{‰}$). EtOH was mixed with water at concentrations of 0.5, 1, 2, 4, and 8%
 160 (vol./vol.) and MeOH at 0.1, 0.2, 0.4, 0.8, and 1.6% (vol./vol.). An additional set of
 161 dilutions was prepared combining both compounds at two concentrations (2 and 8%
 162 EtOH with 0.4 and 1.6% MeOH). The same set of mixtures was used to fit linear
 163 regressions for 1) predicting the error associated with varying contaminant
 164 concentration, as proposed in earlier studies (Brand *et al.*, 2009; Schultz *et al.*, 2011;
 165 Leen *et al.*, 2012), and 2) estimating MeOH-equivalent and EtOH-equivalent
 166 concentrations in natural samples. For this purpose, we took the unitless contaminant
 167 levels determined by ChemCorrect™ and identified as 'ORGANIC_MEOH_AMPL'
 168 (for MeOH) and 'ORGANIC_BASE' (for EtOH) in the raw output files (.csv).

169

170 **Isotopic analyses**

171 We analysed the water isotopes of the xylem and soil samples and of the standard
 172 dilutions by IRMS and IRIS. Isotopic ratios were expressed relative to international
 173 standard (VSMOW, Vienna Standard Mean Ocean Water) in per mil notation (‰) (i.e.
 174 isotopic composition):

175

$$176 \quad \delta^{18}\text{O} \text{ or } \delta^2\text{H} = \left(R_{\text{sample}} / R_{\text{standard}} - 1 \right) \times 1000 \quad (1)$$

177

178 where R_{sample} and R_{standard} are the heavy to light isotopic ratios ($^2\text{H}/\text{H}$ and $^{18}\text{O}/^{16}\text{O}$) of the
 179 sample and the standard, respectively.

180

181 **IRMS methods**

182 We used three different methods for IRMS analysis: (1) $\delta^{18}\text{O}$ and $\delta^2\text{H}$ by high
 183 temperature pyrolysis (labelled as TCEA), conducted at the Paul Scherrer Institute
 184 (Villigen, Switzerland); (2) $\delta^{18}\text{O}$ by CO_2 headspace equilibration using a GasBench II
 185 system (labelled as GB; Thermo Finnigan, Bremen); and (3) $\delta^2\text{H}$ by reduction over
 186 chromium using an H/Device (labelled as HDEV; Thermo Finnigan, Bremen). The
 187 latter two methods were applied at the Center for Stable Isotope Biogeochemistry
 188 (Berkeley, CA, USA). For determining $\delta^{18}\text{O}$ and $\delta^2\text{H}$ by high-temperature pyrolysis (1),

189 a 0.6 μl aliquot of the water sample was injected into a High Temperature Combustion
190 Elemental Analyzer (TC/EA, Thermo Finnigan, Bremen). The water was reduced at
191 1450°C on glassy carbon to H_2 and CO , and these components were then carried in a
192 helium stream to the mass spectrometer (Delta plus XP, Thermo Finnigan, Bremen).
193 The hydrogen isotope ratio was determined from the $^2\text{H}/^1\text{H}$ ratio of the H_2 molecule,
194 and the oxygen isotope ratio was determined from the $^{12}\text{C}^{18}\text{O}/^{12}\text{C}^{16}\text{O}$ ratio of the CO
195 molecule. The precision of this method (1σ standard error of replicates of reference
196 samples) was estimated to be $<0.2\text{‰}$ for $\delta^{18}\text{O}$ and $<1.0\text{‰}$ for $\delta^2\text{H}$. In the GasBench
197 method (2), water samples were equilibrated with a 0.2% CO_2 headspace in Helium for
198 48 h at 21-23°C and later inserted into the GasBench II system connected to the Delta
199 Plus XL mass spectrometer, which measured the $^{18}\text{O}/^{16}\text{O}$ ratio from the CO_2 . The
200 precision was about 0.12‰ for $\delta^{18}\text{O}$. In the chromium combustion method (3),
201 microlitre quantities of water were injected into the H/Device and reduced to H_2 gas.
202 The $^2\text{H}/^1\text{H}$ ratio of this gas was measured by the coupled Delta Plus mass spectrometer.
203 The precision for this method was about 0.80‰ for $\delta^2\text{H}$.

204

205 ***IRIS methods***

206 The IRIS analyses used L2120-*i* and L1102-*i* isotopic water analysers (Picarro Inc.,
207 Sunnyvale, CA, USA) available at the Serveis Científic-Tècnics of the Universitat de
208 Lleida (Lleida, Spain) and at the Center for Stable Isotope Biogeochemistry of the
209 University of California (Berkeley, USA) respectively. The L2120-*i* was coupled to an
210 A0211 high-precision vaporiser, and the L1102-*i* was coupled to a V1102-*i* vaporisation
211 module. One microlitre of water was injected into a vaporisation chamber, and the
212 vapour was then passed into an infrared absorbance cavity. The hydrogen and oxygen
213 isotope ratios were calculated by measuring the decay time of laser light at specific
214 wavelengths on the cavity and by reference to the absorption peaks of the three most
215 abundant isotopologues of water (H_2^{16}O , HD^{16}O , and H_2^{18}O) (Cavity Ring-Down
216 Spectroscopy – CRDS (Gupta *et al.*, 2009)). The estimated precision for the L2120-*i*,
217 based on the repeated analysis of 4 reference water samples was 0.10‰ and 0.40‰, for
218 $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively. The long-term external precision for the L1102-*i* is 0.14‰
219 for $\delta^{18}\text{O}$ and 1.0‰ for $\delta^2\text{H}$.

220

221 **Micro-Combustion Module**

222 After the analysis of water samples with both L2120-*i* and L1102-*i* set with default
 223 settings, the MCM was installed to reanalyse a subset of 79 samples representing most
 224 plant species and soil samples. The MCM was integrated in-line between the Picarro
 225 vaporiser and the L2120-*i* water-isotope analyser at the Serveis Científico-Tècnics of
 226 the Universitat de Lleida. Samples with a small amount of water available after long-
 227 term storage were discarded to avoid potential fractionation effects.

228

229 **Spectral analysis of IRIS data: ChemCorrect™ and post-processing correction**

230 A first quality assessment of the spectral IRIS data was made by running the
 231 PostProcess ChemCorrect™, version 1.2.0 (Picarro Inc., Santa Clara, CA) with
 232 “chemcorrect_inst avg_orgeval_06.csv” instruction file. This software does not perform
 233 corrections on contaminated data but assigns metrics describing the magnitude of the
 234 contamination and its potential source. The software also includes flagging indicating
 235 the degree of potential contamination by a colour code: green for uncontaminated
 236 samples, yellow for possibly contaminated samples (i.e. warranting further attention),
 237 and red for very contaminated samples (i.e. designating unreliable results).

238 As described in Picarro’s forum (link only available for registered users:
 239 http://www.picarro.com/community/picarro_community/applying_corrections_to_contaminated_water_isotope_measurements_using_ch#comment-964) the raw output files
 240 (.csv extension) from the Picarro analysers also provide values of H₂¹⁸O, HD¹⁶O, and
 241 H₂¹⁶O peaks filtered by the spectral features of organic compounds (columns
 242 'ORGANIC_77', 'ORGANIC_82' and 'SPLINEMAX', respectively). The values of the
 243 filtered peaks can be converted to organic-corrected δ¹⁸O and δ²H by applying unit-
 244 specific factory calibration settings (*slope* and *offset*) as:

245

$$247 \quad \delta^2\text{H} = \text{slope} \times \left(\text{HD}^{16}\text{O} / \text{H}_2^{16}\text{O} \right) + \text{offset} \quad (2)$$

$$248 \quad \delta^{18}\text{O} = \text{slope} \times \left(\text{H}_2^{18}\text{O} / \text{H}_2^{16}\text{O} \right) + \text{offset} \quad (3)$$

249 The values for *slope* and *offset* can be found in the file "Picarrocrds.ini" for
 250 Picarro L11xx-*i* units, and the files "InstrCal_Air.ini" and "InstrCal_N2.ini" (measuring
 251 in air and N₂, respectively) for L21xx-*i* units. After including these formulae in a
 252 custom-made Excel spreadsheet, we ended up with two columns with the pre-existing

253 uncorrected values (labelled as 'd(18_16)Mean' and 'd(D_H)Mean' in the original .csv
 254 file), plus two new columns of post-processed values. In both cases, calibration was
 255 then performed by fitting a linear regression to two sets of three internal laboratory
 256 standards included in each batch, using the same custom-made Excel spreadsheet. It
 257 should be noted that we did not use the results from the calibration procedure included
 258 in ChemCorrect™, since this could only be applied to the original, uncorrected values.

259

260 **Data analysis**

261 Differences between IRMS, uncorrected IRIS and post-processed IRIS measurements
 262 were estimated using mixed models based on Restricted Maximum Likelihood (REML)
 263 estimations for both $\delta^{18}\text{O}$ and $\delta^2\text{H}$ ($\alpha = 0.05$). Type of analysis (IRMS, IRIS
 264 uncorrected, IRIS post-processed, MCM and MCM plus post-processing), type of
 265 sample (plant family or soil) and their interaction were considered as fixed factors,
 266 while species within family and sample ID were taken as random factors. The
 267 effectiveness of the different methods in field-collected samples was assessed by the
 268 determination coefficient (R^2) of the linear regression between IRIS and IRMS values,
 269 and the root mean square error (RMSE), calculated as follows:

270

$$271 \quad \text{RMSE} = \sqrt{(Y_{\text{IRIS}} - Y_{\text{IRMS}})^2 / N} \quad (4)$$

272

273 Where Y_{IRIS} and Y_{IRMS} stand for measured IRIS and IRMS, respectively, and N is
 274 the number of samples. Hence, we assumed that IRMS provided “true” values and also
 275 uniform results across IRMS methods. Indeed, a previous study (West *et al.*, 2010)
 276 reported very consistent isotopic ratios among IRMS methods (HDEV, TCEA, and
 277 GB), with discrepancies lower than the range of long-term instrument precision. To
 278 assess the capacity of ChemCorrect™ to flag contaminated samples and to better
 279 understand the limitations of each method, we plotted the differences between IRIS and
 280 IRMS values by plant family and ChemCorrect™ category.

281

282 For the batch of standard dilutions of MeOH and EtOH, the error was directly
 283 calculated as the difference between the measured value of each dilution (both IRIS and
 284 IRMS) and that of pure water analysed by IRMS. As a broad quality threshold for
 285 method comparison, we adopted the values of the maximum accepted bias (MAB)

286 applied in the most recent proficiency test for the analysis of water isotopes coordinated
287 by the Isotope Hydrology Section of the IAEA ($\pm 0.8\text{‰}$ and $\pm 6\text{‰}$ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$,
288 respectively; [http://nucleus.iaea.org/rpst/ReferenceProducts/Proficiency_Tests/IAEA-
289 TEL-2011-01/index.htm](http://nucleus.iaea.org/rpst/ReferenceProducts/Proficiency_Tests/IAEA-TEL-2011-01/index.htm), M. Groening, pers. comm.). Other studies have proposed
290 narrower limits for accuracy on hydrological studies (e.g. $\pm 0.2\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 2\text{‰}$ for
291 $\delta^2\text{H}$, as used in the IAEA inter-laboratory test WICO2011, see Wassenaar *et al.*, 2012 or
292 $\pm 0.15\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 1\text{‰}$ for $\delta^2\text{H}$ in Wassenaar *et al.*, 2014). However, since in our
293 study we compared different methods and different laboratories among them, and not
294 against a reference value, we considered more informative to use a broader threshold as
295 primary assessment. In any case, in order to overcome the limitations associated to the
296 use of arbitrary thresholds to identify a proper methodology we also assessed the
297 distribution of errors among the samples using a histogram with 0.2‰ and 2‰ classes
298 for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively. Statistical analyses were performed with JMP Pro 11
299 (SAS Inc., Cary, NC, USA).

300

301 **RESULTS**

302

303 **Effect of contaminants on water isotopic composition and correction methods:**
304 **ethanol- and methanol-water mixtures**

305 Table 2 shows the range of deviations of IRIS and IRMS values from IRMS analysis of
306 pure water (used as reference value) for the set of mixtures. The errors associated with
307 mixtures at different MeOH and EtOH concentrations are shown in Fig. 1. MeOH/water
308 mixtures analysed by IRIS differed substantially from the reference value starting at the
309 lowest contaminant concentration (0.1% MeOH), with maximum discrepancies between
310 the uncorrected IRIS and the IRMS reference value as large as -142.96 and -1077‰ for
311 $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively. In contrast, EtOH did not interfere as strongly with pure
312 water, even at very high concentrations (up to 8%). Maximum differences were -0.39‰
313 for $\delta^{18}\text{O}$ and -10.76‰ for $\delta^2\text{H}$. The error exceeded the established maximum bias for
314 $\delta^2\text{H}$ only at concentrations of 8%. Similarly, the interferences caused by MeOH and
315 EtOH mixtures on water isotopic signatures were mostly due to MeOH, as any
316 particular combination of EtOH and MeOH produced a deviation in isotopic signatures
317 similar to that using MeOH alone. The maximum errors for EtOH and MeOH mixtures
318 (-147.06‰ for $\delta^{18}\text{O}$ and -1104.64‰ for $\delta^2\text{H}$ at 1.6% MeOH and 2% EtOH) were thus
319 comparable to the error for the highest MeOH concentration (1.6% MeOH). In contrast,
320 MeOH caused negligible effects on IRMS values within the range of concentrations
321 used, whereas we found larger errors for IRMS than for IRIS with EtOH concentrations
322 starting at 4% for $\delta^{18}\text{O}$ and 1% for $\delta^2\text{H}$.

323 The post-processing correction of contaminant interference for L2110-*i* and
324 L2120-*i* reallocated the IRIS values within threshold limits (± 0.8 and $\pm 6\%$ for $\delta^{18}\text{O}$ and
325 $\delta^2\text{H}$, respectively) for MeOH concentrations below 0.8% for $\delta^{18}\text{O}$ and 0.2% for $\delta^2\text{H}$.
326 For EtOH, the correction always increased analytical accuracy, even though uncorrected
327 values were usually within MAB limits (Fig. 1). The removal of organic interferences
328 by the MCM improved the accuracy of IRIS values for both isotopes in the MeOH
329 dilutions for contaminant concentrations up to 0.8%, but for EtOH dilutions the MCM
330 tended to produce larger errors than the non-treated IRIS for concentrations $\geq 2\%$. In
331 mixed dilutions, the MCM was clearly influenced by the quantity of EtOH at equal
332 MeOH concentrations (Fig. 1). The effect of small amounts of residual MeOH after

333 MCM pre-treatment in the highest concentration levels was generally corrected by post-
334 processing, but the treatment of EtOH produced overcorrected values (Fig. 1).

335

336 **Effect of contaminants on water isotopic composition and correction methods:**
337 **natural samples**

338 We found significant differences in isotopic compositions between uncorrected IRIS
339 and IRMS values for the complete set of 136 samples analysed (Table 3). The
340 maximum discrepancies between methods were -17.25‰ ($\delta^{18}\text{O}$) and -78.08‰ ($\delta^2\text{H}$) for
341 soil samples, and -8.34‰ ($\delta^{18}\text{O}$) and -92.19‰ ($\delta^2\text{H}$) for xylem samples. In particular,
342 20% ($\delta^{18}\text{O}$) and 22% ($\delta^2\text{H}$) of the samples fell outside the limits of the MAB, and about
343 10% showed very strong negative deviations (below -2‰ and -20‰, for $\delta^{18}\text{O}$ and $\delta^2\text{H}$,
344 respectively, see Fig. 2a,b). After post-processing, differences in isotopic compositions
345 between IRIS and IRMS values were still significant for $\delta^{18}\text{O}$ but became non-
346 significant for $\delta^2\text{H}$ (Table 3). The maximum differences were -1.79‰ for $\delta^{18}\text{O}$ and
347 +26.74‰ for $\delta^2\text{H}$ in soil samples and +1.76‰ for $\delta^{18}\text{O}$ and +8.55‰ for $\delta^2\text{H}$ in xylem
348 samples. Overall, the number of samples outside the MAB decreased to 7% ($\delta^{18}\text{O}$) and
349 4% ($\delta^2\text{H}$). Deviations from IRMS values produced a slight (although within MAB
350 limits) positive bias (Fig. 2c,d).

351 Considering only the subset of 79 samples reanalysed with the MCM, we also
352 found significant differences in isotopic compositions between uncorrected IRIS and
353 IRMS values (Table 3). Within this subset, 29% ($\delta^{18}\text{O}$) and 27% ($\delta^2\text{H}$) of the samples
354 were originally outside the threshold values of the MAB. This percentage decreased to
355 9% ($\delta^{18}\text{O}$) and 4% ($\delta^2\text{H}$) after post-processing correction and to 5% ($\delta^{18}\text{O}$) and 6%
356 ($\delta^2\text{H}$) with MCM pre-treatment (IRIS plus MCM, Table 2). In this regard, there were no
357 significant differences between the pre-treatment and the software correction methods,
358 both being statistically equivalent to IRMS. Besides, we also did not find significant
359 differences between post-processing correction after MCM operation (IRIS plus MCM
360 post-processed) and the other combinations (IRIS post-processed and IRIS plus MCM
361 alone) (Table 3). However, the MCM pre-treatment produced a larger number of
362 samples having systematic positive errors (although still within MAB limits) than the
363 post-processing correction (Fig. 2e,f), resulting in a histogram clearly biased to positive
364 values, particularly for $\delta^{18}\text{O}$. For the MCM with post-processing, differences in isotopic
365 compositions between IRIS and IRMS were slightly higher than those without post-

366 processing, but in the range of the other two combinations, with 10% ($\delta^{18}\text{O}$) and 6%
 367 ($\delta^2\text{H}$) of samples outside the MAB and similar positive bias (Fig. 2g,h).

368 The correction based on linear regression of the water mixtures was less
 369 successful than the post-processing correction or the removal of organics by MCM. For
 370 the MeOH concentration only ('ORGANIC_MEOH_AMPL' column in the raw Picarro
 371 output files), the regression-based correction placed 13% ($\delta^{18}\text{O}$) and 26% ($\delta^2\text{H}$) of
 372 collected samples outside the MAB. Adding a second correction based on EtOH
 373 concentration ('ORGANIC_BASE' column) produced very similar results (data not
 374 shown).

375

376 **Relationships between IRMS- and IRIS-based approaches for stable isotopes in** 377 **water**

378 Fig. 3a,b compares IRMS and IRIS values (before and after post-processing correction)
 379 for the entire dataset ($N=136$). Goodness-of-fit statistics (R^2 and root mean square error,
 380 RMSE) of the linear regressions between IRIS and IRMS indicated that the post-
 381 processing correction eliminated most discrepancies due to organic interference, even
 382 for highly contaminated samples.

383 Table 4 shows the statistics of the linear regressions between IRIS and IRMS
 384 values for the subset of samples analysed with the MCM for each category of
 385 ChemCorrect™ contamination. An important improvement in R^2 and a concomitant
 386 decrease in the RMSE were observed after activating the MCM, indicating an effective
 387 removal of interferences caused by contamination with organics. Considering the
 388 ChemCorrect™ categories, R^2 increased from 0.06 to 0.89 ($\delta^{18}\text{O}$) and from 0 to 0.88
 389 ($\delta^2\text{H}$) for the red-flagged samples. For the yellow-flagged samples, R^2 increased from
 390 0.69 to 1 ($\delta^{18}\text{O}$) and from 0.69 to 0.99 ($\delta^2\text{H}$). Moreover, 43 and 83% of the samples first
 391 flagged as yellow and red, respectively, were classified as green after MCM operation.

392

393 **Elimination of contaminants by the MCM**

394 We found a strong correspondence between known alcohol concentrations and
 395 ChemCorrect™ quantification values for MeOH ($R^2 = 0.99$) and EtOH ($R^2 = 0.99$). In
 396 our set of mixtures, 1% MeOH corresponded to approximately 0.1 units in the
 397 'ORGANIC_MEOH_AMPL' column and 1% EtOH corresponded to approximately
 398 245 units in the 'ORGANIC_BASE' column. We applied these equivalences in order to

399 compare the effectiveness of the MCM to remove contaminants in alcohol-water
400 mixtures and natural samples. Mean values for equivalent MeOH and EtOH (%)
401 concentration for each family and ChemCorrect™ flagging category are shown in Fig.
402 4. Equivalent MeOH concentrations in samples ranged from 0 to 0.06% for xylem
403 water, and from 0 to 0.32% for soil samples. Equivalent EtOH concentrations ranged
404 from 0 to 6.7% in the xylem, and from 0 to 0.03% for soil samples. These values were
405 within the range of the set of standard dilutions (0.1-1.6% for MeOH and 0.5-8% for
406 EtOH). MeOH was nearly completely eliminated by the MCM in both the natural
407 samples and the set of mixtures; the estimated maximum residual concentration was
408 0.01% for samples and up to 0.09% for 1.6% MeOH dilutions. In contrast, the MCM
409 was more effective at removing the EtOH from the artificial mixtures than at
410 eliminating 'C₂₊ alcohols' in soil and xylem samples. Despite having higher initial
411 concentrations, the residual EtOH-equivalent concentration was about one order of
412 magnitude lower in the mixtures (mean, 0.016%; maximum, 0.03%) than in the samples
413 (mean, 0.17%; maximum, 3.9%; see Fig. 4). The higher residual concentrations in
414 samples, however, did not produce higher deviations from IRMS values (compare Fig. 1
415 for artificial mixtures with Figs. 5 and 6 for the natural samples).

416

417 **Contaminant effects among plant families**

418 Figs. 5a,b,c,d and 6a,b,c,d illustrate the differences in isotopic compositions ($\delta^{18}\text{O}$ and
419 $\delta^2\text{H}$, respectively) between IRIS and IRMS values ($\delta_{\text{IRIS}} - \delta_{\text{IRMS}}$) among plant families.
420 These differences were generally negative in the most contaminated samples (see Fig.
421 4). Both MCM operation and post-processing correction increased the agreement
422 between IRIS and IRMS and reallocated most samples within the established MAB
423 threshold. The MCM, however, produced a systematic positive bias in $\delta_{\text{IRIS}} - \delta_{\text{IRMS}}$
424 differences in almost all plant families.

425

426 **DISCUSSION**

427

428 **A simple IRIS post-processing reduces contaminant interference**

429 As previously reported, the isotopic composition of some natural samples analysed by
430 IRIS showed strong negative deviations from IRMS values (Brand *et al.*, 2009; West *et al.*,
431 2010, 2011; Zhao *et al.*, 2011; Schmidt *et al.*, 2012). Differences were particularly
432 high for soil samples as compared to other studies (West *et al.*, 2010; Zhao *et al.*, 2011),
433 being in the range of previously published values for xylem samples (West *et al.*, 2010,
434 2011; Zhao *et al.*, 2011; Schmidt *et al.*, 2012). Nevertheless, it should be noted that the
435 most contaminated samples (differences $< -2\%$ in $\delta^{18}\text{O}$) corresponded to downhill and
436 valley-bottom soils in a gypsum-rich area, characterized by the accumulation of solutes
437 and mineral nutrients, contrasting with the limited nutrient availability in the top of the
438 hills (Guerrero-Campo *et al.*, 1999; Palacio *et al.*, 2014b). Hence, the potential
439 interference of electrolytes in soils with IRIS measurements may require a more detailed
440 assessment.

441 The post-processing correction proposed by Picarro strongly reduced the effects
442 of contamination, even in cases of heavily contaminated samples. As expected, the
443 correction limits for MeOH were relatively low due to its strong spectral interference
444 (deviations were within MAB up to concentrations of 0.4% and 0.1% MeOH for $\delta^{18}\text{O}$
445 and $\delta^2\text{H}$, respectively). Conversely, the deviation of corrected values was below the
446 MAB even at the highest tested concentration of EtOH (8%). For xylem and soil
447 samples, the post-processing correction reduced the discrepancies between IRIS and
448 IRMS from RMSEs of 2.42‰ to 0.42‰ for $\delta^{18}\text{O}$ and of 18.46‰ to 3.95‰ for $\delta^2\text{H}$, and
449 reallocated more than 70% of highly deviating samples within MAB limits.
450 Nevertheless, a closer look at the error distribution (Fig. 2c,d) reveals a positive error
451 bias of about 0.2‰ for $\delta^{18}\text{O}$ and 2‰ for $\delta^2\text{H}$. This bias, however, is in the range of the
452 expected additive effect of laboratory uncertainties and potential sample alteration
453 during transport and storage. In fact, we also found slightly positive differences between
454 IRIS and IRMS for the pure water samples used for the set of alcohol-water mixtures
455 (up to +0.36‰ for $\delta^{18}\text{O}$, and +0.89‰ for $\delta^2\text{H}$, see Fig. 1). In this regard, our results
456 show the potential of post-processing correction methods as a way to solve contaminant
457 issues for IRIS, but also encourage a more exhaustive assessment of their accuracy, e.g.
458 following the robust procedures of global inter-laboratory tests.

459

460 MCM: effectiveness and limitations

461 An overall reduction in the maximum differences between IRIS and IRMS values in the
462 natural samples was obtained with the MCM in operation, even for highly contaminated
463 samples. The RMSE decreased to 0.54‰ for $\delta^{18}\text{O}$ and 3.52‰ for $\delta^2\text{H}$. More than 75%
464 of samples initially placed outside the MAB fell within this threshold after using the
465 MCM. The post-processing correction and the MCM were generally equally effective
466 for $\delta^{18}\text{O}$ analysis in the presence of MeOH contamination, but the post-processing
467 correction was less precise for $\delta^2\text{H}$ (Table 4). Indeed, when methanol was the main
468 contaminant (as in methanol-water mixtures and in contaminated soil samples; Fig. 4a),
469 MCM seemed to outperform the post-processing correction (Fig. 1, Fig. 5b,c, and Fig.
470 6b,c). In contrast, the post-processing correction was consistently more effective than
471 the MCM at removing errors associated with C_{2+} alcohols such as EtOH (see Fig. 1).
472 Furthermore, using the MCM a substantial proportion of samples showed positive
473 deviations between 0.4‰ and 0.8‰ for $\delta^{18}\text{O}$, and between 2‰ and 6‰ for $\delta^2\text{H}$ (Fig.
474 2e,f). This positive bias is likely to be a collateral effect of the contaminant removal.
475 MCM oxidation converts organic compounds into CO_2 and nascent water by using an
476 air carrier gas supported by ambient O_2 . For each EtOH molecule, the MCM generates
477 three water molecules that mix with the water in the sample. In this reaction the
478 hydrogen atoms originate from the alcohol, whereas the oxygen mostly comes from the
479 carrier gas ($\delta^{18}\text{O}_{\text{air}} = +23.8 \pm 0.3\text{‰}$ (Coplen *et al.*, 2002)). If the alcohol content in the
480 sample is sizeable, the MCM significantly alters the isotopic signature of the water
481 proportionally to (i) the relative mass contribution of the hydrogen and oxygen atoms of
482 the sample water and that of the water formed through chemical oxidation of alcohols,
483 and (ii) their corresponding isotopic signatures. Our results were consistent with this
484 expectation, with more biased values for $\delta^{18}\text{O}$ than for $\delta^2\text{H}$ analysed by the MCM in
485 comparison to IRMS, due to the very positive oxygen isotopic composition of air. Large
486 errors can consequently be generated at high concentrations of organic contaminants in
487 the samples because the oxidation process adds new water molecules to the water pool,
488 despite effectively reducing spectral interference. We would thus recommend post-
489 processing correction instead of MCM operation when analysing samples of unknown
490 composition or with expectedly high concentrations of EtOH and longer-chain alcohols.

491 The MCM nearly completely eliminated MeOH and EtOH from the artificial
492 mixtures, but was less effective at removing the C_{2+} alcohols pool from natural samples

493 (presumably including ethanol derived from anaerobic metabolism, terpenols and other
494 volatiles, see refs. in (Niinemets U. and Monson R.K. (eds.), 2013). Despite this, the
495 artificial mixtures still produced divergences beyond the MAB for concentrations above
496 0.4% MeOH and 2% EtOH, due to the side-effects of the oxidation process. Conversely,
497 we could not establish a clear threshold for natural samples based on estimated
498 contaminant concentration (Fig. 4). Although the concentrations of C₂₊ alcohols
499 remaining after MCM operation were higher in the natural samples than in the mixtures,
500 the spectral interference was significantly lower in the samples. This could be attributed
501 to a limited interference of other C₂₊ alcohols as compared to EtOH.

502 We also tested the possibility of applying post-processing correction to samples
503 previously treated by micro-combustion to improve the performance of the MCM. The
504 post-processing correction, however, apparently overcorrected the isotopic values, with
505 the exception of highly contaminated samples with residual MeOH (see Fig. 2g,h). The
506 MCM was designed to remove the spectral interference of organic compounds at low
507 concentrations. In samples with high concentrations of contaminants (e.g. EtOH
508 dilutions) the organic interference is effectively removed by the MCM, but at the
509 expense of altering the isotope composition of water. Hence, although post-processing
510 may still correct the spectral interferences caused by remaining alcohols after MCM
511 operation, the resulting “corrected” values will be those of the isotopically-altered
512 water. The development of an integrated post-processing correction would thus be
513 advisable (e.g. considering spectral information before and after MCM operation) as a
514 way to account for changes in water isotope composition caused by the MCM.

515

516 **Spectral post-processing outperforms previously proposed empirical corrections**

517 Previous studies have proposed correction curves as a function of the degree of
518 contaminant concentration (Brand *et al.*, 2009; Schultz *et al.*, 2011; Leen *et al.*, 2012).
519 Schultz *et al.* (2011) eliminated (for $\delta^{18}\text{O}$) or reduced (for $\delta^2\text{H}$) the discrepancies
520 between IRIS and IRMS results using an LGR Liquid Water Isotope Analyzer (Los
521 Gatos Research Inc., Mountain View, CA). The recommended curves, however, did not
522 match those provided by the manufacturer, so the authors suggested that every analyser
523 could require a customized correction. Brand *et al.* (2009) also performed regressions of
524 δ -values and contaminant concentrations for a set of standard dilutions and concluded
525 that corrections of isotopic values are feasible provided the alcohol content in the
526 samples is known. We consequently corrected the isotopic values by using linear

527 regressions to predict IRIS δ -errors for pure water as a function of contaminant
528 concentration according to the CH₃OH and C₂₊ alcohol outputs from ChemCorrect™.
529 The precision however, was lower than that obtained through post-processing
530 correction, and the most contaminated samples were usually extremely overcorrected,
531 resulting in very high isotopic values. Post-processing correction based on peaks filtered
532 by ChemCorrect™ seems thus a more suitable alternative than the correction based on
533 organic concentrations in samples.

534 In spite of this, corrections based on estimated MeOH concentrations still
535 improved the accuracy of isotopic records, but the calibrations performed with the
536 EtOH dilutions did not work well for natural samples. This could be due to the fact that
537 MeOH concentration can be specifically quantified based on a well-defined peak,
538 whereas EtOH produces mainly a baseline drift in the spectra, and is measured together
539 with a pool of long-chain alcohols. Similarly, Brand *et al.* (2009) found no relationship
540 between EtOH concentration and δ -value in wine due to interference from contaminants
541 such as MeOH, phenols, or organic acids.

542

543 **Concluding remarks**

544 According to our results, the post-processing correction of isotope values based on
545 spectral analyses improves significantly the performance of IRIS in soil and xylem
546 samples, thus allowing detailed ecohydrological studies at a reasonable cost. In
547 particular, differences between IRMS and IRIS-corrected values fell within reasonable
548 limits in most field-collected samples (>90%). According to our dilution tests,
549 interferences associated to organic contaminants can be successfully removed with
550 concentrations up to 8% and 0.4% for EtOH and MeOH, respectively. Sample pre-
551 treatment through the MCM slightly outperforms post-processing correction in
552 removing MeOH interference. Nevertheless, for heavily MeOH-contaminated samples,
553 the best results would be obtained combining both methods, which together may be able
554 to correct samples with up to 1.6% MeOH contamination. In contrast, the MCM was not
555 effective in removing EtOH interference: with high concentrations of contaminant the
556 module causes significant changes in the isotope composition of water (particularly
557 strong for $\delta^{18}\text{O}$). Hence, for contaminated samples we generally recommend to adopt
558 post-processing correction in isotopic analyses, and only when the main (and mostly

559 unique) contaminant detected is MeOH (as in our soil samples), the use of MCM
560 (eventually combined with post-processing correction).

561

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574

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- 678

679 **FIGURE LEGENDS**

680

681 **Fig. 1** Errors for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ (‰) associated with various methanol (MeOH) and
 682 ethanol (EtOH) concentrations in the alcohol-water mixtures. The errors have been
 683 calculated as the differences between the dilutions and pure water analysed by IRMS.
 684 Dashed lines represent the accuracy thresholds based on the maximum accepted bias
 685 (MAB) established by the International Agency of Atomic Energy.

686

687 **Fig. 2** Histogram representing the distribution of deviations between IRIS and IRMS for
 688 $\delta^{18}\text{O}$ (left panels) and $\delta^2\text{H}$ (right panels). (a,b) IRIS uncorrected; (c,d) IRIS post-
 689 processed; (e,f) IRIS plus MCM; (g,h) IRIS plus MCM plus post-processing. The
 690 samples were grouped into 0.2‰ and 2‰ intervals, for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively. For
 691 simplicity, heavily deviated samples (outside the [-2‰ +2‰] range for $\delta^{18}\text{O}$ and the [-
 692 20‰ +20‰] range for $\delta^2\text{H}$) were included in a single bin. Light grey: whole dataset.
 693 Dark grey: subsample used for the assessment of the MCM.

694

695 **Fig. 3** Comparison between IRIS and IRMS for uncorrected (filled symbols) and post-
 696 processed (empty symbols) $\delta^{18}\text{O}$ values (a) and $\delta^2\text{H}$ values (b) using 136 field samples.
 697 Linear regression equations, R^2 and RMSEs are also presented. Dotted lines represent
 698 95% confidence intervals. Circles, xylem samples; Triangles, soil samples.

699

700 **Fig. 4** Estimated equivalent concentrations (%) of methanol (MeOH, upper panels) and
 701 ethanol (EtOH, lower panels) by plant family, determined by fitting the spectral
 702 information provided by the Chemcorrect™ (respectively, 'ORGANIC_MEOH_AMPL'
 703 and 'ORGANIC_BASE' columns in the raw output files) against known values of
 704 MeOH and EtOH in the alcohol-water mixtures. Within each family, three coloured bars
 705 indicate the flagging categories of ChemCorrect™. Left and right panels show raw
 706 sample concentrations and concentrations after MCM pre-treatment, respectively.

707

708 **Fig. 5** Differences in $\delta^{18}\text{O}$ values between IRIS and IRMS by plant family. IRIS
 709 uncorrected (a), IRIS post-processed (b), IRIS plus MCM (c) and IRIS plus MCM plus
 710 post-processing (d). Error bars are standard errors. Dashed lines identify the maximum

711 accepted bias (MAB) established by the International Agency of Atomic Energy.
712 Colour codes represent the flagging categories of ChemCorrect™.

713

714 **Fig. 6** Differences in $\delta^2\text{H}$ values between IRIS and IRMS by plant family. IRIS
715 uncorrected (a), IRIS post-processed (b), IRIS plus MCM (c) and IRIS plus MCM plus
716 post-processing (d). Error bars are standard errors. Dashed lines identify the maximum
717 accepted bias (MAB) established by the International Agency of Atomic Energy.
718 Colour codes represent the flagging categories of ChemCorrect™.

719

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720

Table 1. Description of the plant species and soil samples used in this study. Soil description according to FAO classification.

721

Species/Soil type	Family	Season	Origin	No. of samples	No. of samples in the MCM subset
<i>Arbutus unedo</i> L.	Ericaceae	Fall, winter & summer	Catalonia	5	3
<i>Artemisia herba-alba</i> Asso	Asteraceae	Spring	Aragon	1	1
<i>Baccharis pilularis</i> DC.	Asteraceae	Fall	California	7	5
<i>Buxus sempervirens</i> L.	Buxaceae	Summer	Aragon	3	3
<i>Cistus clusii</i> Dunal	Cistaceae	Fall	Murcia	3	0
<i>Erica arborea</i> L.	Ericaceae	Winter & summer	Catalonia	4	2
<i>Erica multiflora</i> L.	Ericaceae	Fall, winter & summer	Valencia	5	1
<i>Fagus sylvatica</i> L.	Fagaceae	Summer	Catalonia	1	1
<i>Helianthemum squamatum</i> (L.) Dum. Cours.	Cistaceae	Spring	Aragon	4	4
<i>Lepidium subulatum</i> L.	Brassicaceae	Spring	Aragon	3	3
<i>Linum suffruticosum</i> L.	Linaceae	Spring	Aragon	1	1
<i>Stipa tenacissima</i> L.	Poaceae	Fall & spring	Andalusia	3	2
<i>Phillyrea latifolia</i> L.	Oleaceae	Fall, winter & summer	Catalonia	4	1
<i>Phlomis purpurea</i> sub. <i>almeriensis</i> Pau	Lamiaceae	Fall	Andalusia	2	0
<i>Pinus halepensis</i> Mill.	Pinaceae	Fall	Murcia	2	0
<i>Pinus sylvestris</i> L.	Pinaceae	Summer	Aragon	4	4
<i>Pistacia lentiscus</i> L.	Chenopodiaceae	Winter	Catalonia	2	1
<i>Quercus agrifolia</i> Née	Fagaceae	Fall	California	3	3
<i>Quercus coccifera</i> L.	Fagaceae	Winter	Catalonia	1	0
<i>Quercus douglasii</i> Hook. & Arn.	Fagaceae	Fall	California	3	1
<i>Quercus ilex</i> L.	Fagaceae	All	Catalonia	13	6
<i>Quercus kelloggii</i> Newb.	Fagaceae	Fall	California	9	6
<i>Quercus lobata</i> Née	Fagaceae	Fall	California	14	7
<i>Quercus subpyrenaica</i> Villar	Fagaceae	Summer	Aragon	4	4
<i>Suaeda pruinosa</i> Lange	Chenopodiaceae	Spring	Aragon	1	1
<i>Umbellularia californica</i> Hook. & Arn.	Lauraceae	Fall	California	5	4
TOTAL xylem				107	64
Calcaric Leptosol		Winter & summer	Catalonia	5	3
Calcic Cambisol		Summer	Aragon	2	2
Dystric Cambisol		Spring	Catalonia	5	0
Dystric Leptosol		Spring & summer	Catalonia	7	0
Gypsic Regosol		Spring	Aragon	4	4
Gypsisol/Solonchak		Spring	Aragon	6	6
TOTAL soil				29	15
TOTAL				136	79

722

723 **Table 2.** Range (minimum value; maximum value) of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ discrepancies between IRIS and IRMS, and number of samples within the
 724 maximum accepted bias (MAB) used in the last proficiency test of the International Atomic Energy Agency ($\pm 0.8\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 6\text{‰}$ for $\delta^2\text{H}$,
 725 IAEA-TEL-2011-01, see text for details) for IRIS, IRIS plus post-processing correction, and IRIS plus the MCM (either uncorrected or post-
 726 processed) in standard dilutions and the subset ($N=79$) of xylem and soil water samples.

IRIS									
Sample type	No. total samples	Uncorrected				Post-processed			
		Error $\delta^{18}\text{O}$ (‰)	No. within MAB	Error $\delta^2\text{H}$ (‰)	No. within MAB	Error $\delta^{18}\text{O}$ (‰)	No. within MAB	Error $\delta^2\text{H}$ (‰)	No. within MAB
Standard dilutions									
MeOH	5	(-142.96; -8.64)	0	(-1077.00; -64.58)	0	(-2.15; -0.01)	3	(3.35; 44.39)	1
EtOH	5	(-0.39; 0.20)	5	(-10.76; 0.49)	4	(0.13; 0.46)	5	(-4.38; 0.56)	5
MeOH+EtOH	4	(-147.06; -39.65)	0	(-1104.64; -298.72)	0	(-2.29; -0.38)	2	(6.98; 45.39)	0
Collected samples									
Xylem	64	(-8.34; 0.85)	48	(-92.19; 6.17)	50	(-0.33; 1.43)	60	(-1.01; 8.55)	62
Soil	15	(-17.25; 0.24)	8	(-78.08; 4.99)	8	(-1.79; 0.49)	12	(0.13; 6.66)	14
IRIS plus MCM									
Sample type	No. total samples	Uncorrected				Post-processed			
		Error $\delta^{18}\text{O}$ (‰)	No. within MAB	Error $\delta^2\text{H}$ (‰)	No. within MAB	Error $\delta^{18}\text{O}$ (‰)	No. within MAB	Error $\delta^2\text{H}$ (‰)	No. within MAB
Standard dilutions									
MeOH	5	(-8.61; 0.41)	3	(-77.01; 1.21)	3	(0.31; 0.90)	4	(-2.96; 1.90)	5
EtOH	5	(0.22; 2.08)	3	(-19.45; -0.59)	3	(0.41; 2.48)	3	(-17.24; -0.07)	3
MeOH+EtOH	4	(0.31; 2.44)	2	(-23.08; -10.27)	0	(1.11; 3.08)	0	(-19.38; -4.64)	1
Collected samples									
Xylem	64	(-0.92; 1.21)	60	(-8.97; 8.78)	60	(0.01; 1.36)	56	(0.57; 8.91)	60
Soil	15	(-0.09; 0.79)	15	(0.16; 6.5)	14	(-0.09; 0.8)	15	(0.4; 7.45)	14

Table 3. *P*-values and *F*-ratio of the statistical comparisons ($\alpha = 0.05$, mixed models based on Restricted Maximum Likelihood, REML) between correction methods for the complete dataset ($N = 136$) and a subset of natural samples ($N = 79$) for both $\delta^{18}\text{O}$ and $\delta^2\text{H}$.

		$\delta^{18}\text{O}$		$\delta^2\text{H}$	
		<i>F</i> -ratio	<i>P</i> -value	<i>F</i> -ratio	<i>P</i> -value
Complete dataset <i>N</i> =136	IRMS vs. IRIS uncorrected	9.6875	0.0061 *	4.5324	0.0390 *
	IRIS post-processed vs. IRIS uncorrected	29.4845	<0.0001 *	8.2688	0.0062 *
	IRMS vs. IRIS post-processed	5.3707	0.0327 *	0.5574	0.4593
Subset <i>N</i> =79	IRMS vs. IRIS uncorrected	4.2368	0.0443 *	4.7005	0.0341 *
	IRIS post-processed vs. IRIS uncorrected	6.6844	0.0124 *	8.3796	0.0053 *
	IRIS+MCM vs. IRIS uncorrected	8.7293	0.0046 *	8.0996	0.0060 *
	IRMS vs. IRIS post-processed	0.2778	0.6002	0.5281	0.4702
	IRMS vs. IRIS+MCM	0.8032	0.374	0.4596	0.5004
	IRIS post-processed vs. IRIS+MCM	0.1363	0.7134	0.0024	0.9613
	IRIS+MCM vs. IRIS+MCM post-processed	0.1213	0.7289	0.1488	0.7010

732 **Table 4.** Summary statistics of the relationship between IRMS and IRIS values for the subset of natural samples analysed with the MCM ($N=79$)
 733 within each ChemCorrect™ contamination categories. N , Number of samples, R^2 coefficient of determination for the linear regression between
 734 IRIS and IRMS, RMSE (%); Root mean square error of the difference between IRMS and IRIS values.

		ChemCorrect Category			Green			Yellow			Red			All		
		IRMS-IRIS Linear regression			N	R^2	RMSE	N	R^2	RMSE	N	R^2	RMSE	N	R^2	RMSE
$\delta^{18}\text{O}$	IRIS	37	0.97	0.53	13	0.69	1.43	29	0.06	5.09	79	0.31	3.16			
	IRIS post-processed		0.99	0.27		0.97	0.54		0.92	0.56		0.97	0.44			
	IRIS+MCM	56	0.99	0.54	11	1.00	0.58	12	0.89	0.50	79	0.98	0.54			
	IRIS+MCM post-processed		0.99	0.58		1.00	0.62		0.98	0.75		0.99	0.62			
$\delta^2\text{H}$	IRIS	37	0.89	3.33	13	0.69	5.87	29	0.00	38.34	79	0.05	23.46			
	IRIS post-processed		0.98	2.45		0.96	2.50		0.95	3.60		0.96	2.93			
	IRIS+MCM	56	0.97	3.55	11	0.99	2.62	12	0.88	4.05	79	0.93	3.52			
	IRIS+MCM post-processed		0.97	4.00		0.98	3.06		0.99	3.55		0.97	3.82			

735

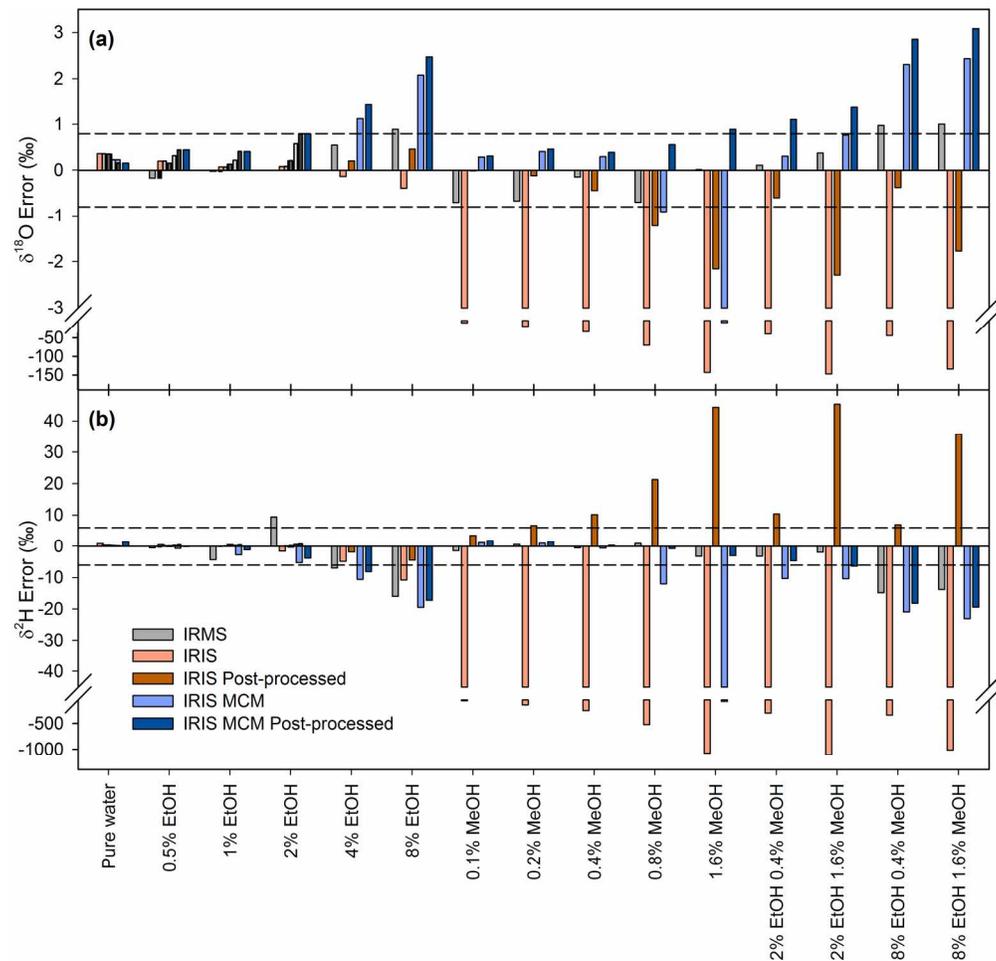


Fig. 1 Errors for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ (‰) associated with various methanol (MeOH) and ethanol (EtOH) concentrations in the alcohol-water mixtures. The errors have been calculated as the differences between the dilutions and pure water analysed by IRMS. Dashed lines represent the accuracy thresholds based on the maximum accepted bias (MAB) established by the International Agency of Atomic Energy. 189x182mm (300 x 300 DPI)

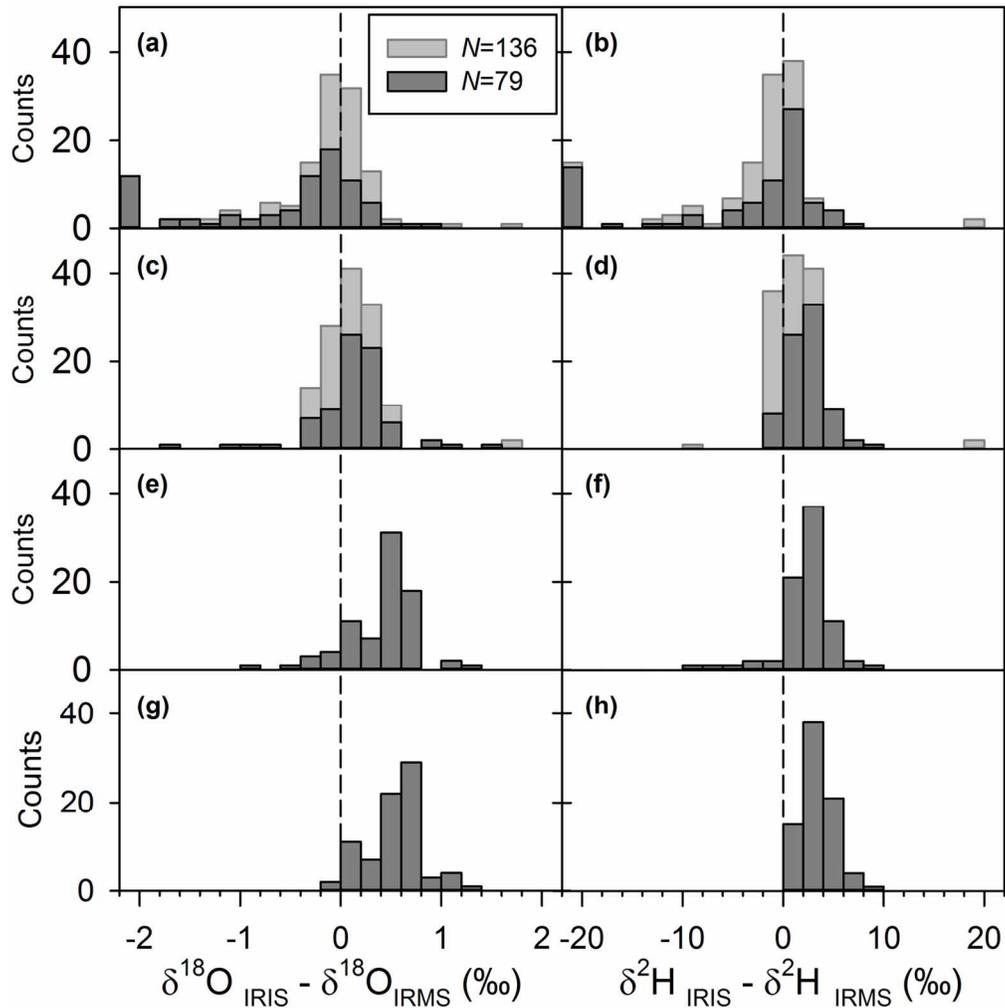


Fig. 2 Histogram representing the distribution of deviations between IRIS and IRMS for $\delta^{18}\text{O}$ (left panels) and $\delta^2\text{H}$ (right panels). (a,b) IRIS uncorrected; (c,d) IRIS post-processed; (e,f) IRIS plus MCM; (g,h) IRIS plus MCM plus post-processing. The samples were grouped into 0.2‰ and 2‰ intervals, for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively. For simplicity, heavily deviated samples (outside the $[-2\text{‰} + 2\text{‰}]$ range for $\delta^{18}\text{O}$ and the $[-20\text{‰} + 20\text{‰}]$ range for $\delta^2\text{H}$) were included in a single bin. Light grey: whole dataset. Dark grey: subsample used for the assessment of the MCM.

136x138mm (300 x 300 DPI)

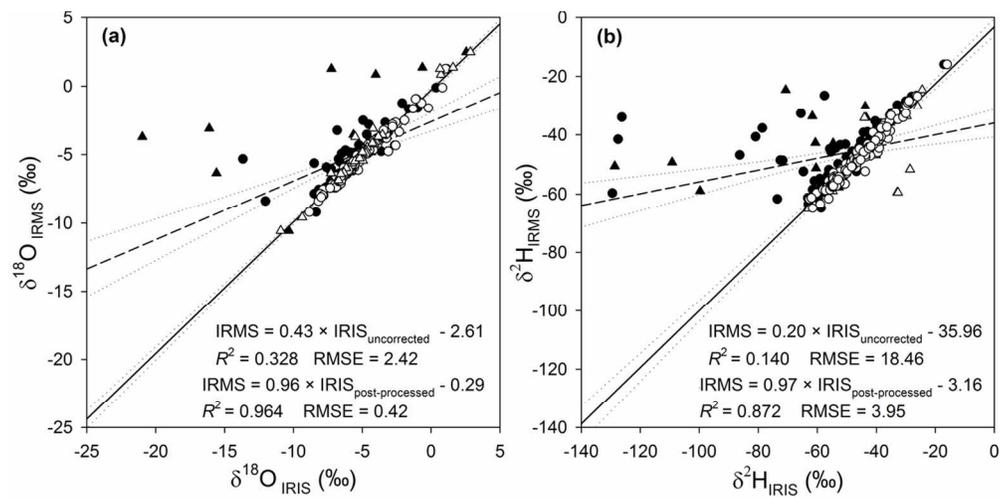


Fig. 3 Comparison between IRIS and IRMS for uncorrected (filled symbols) and post-processed (empty symbols) $\delta^{18}\text{O}$ values (a) and $\delta^2\text{H}$ values (b) using 136 field samples. Linear regression equations, R^2 and RMSEs are also presented. Dotted lines represent 95% confidence intervals. Circles, xylem samples; Triangles, soil samples.

118x58mm (300 x 300 DPI)

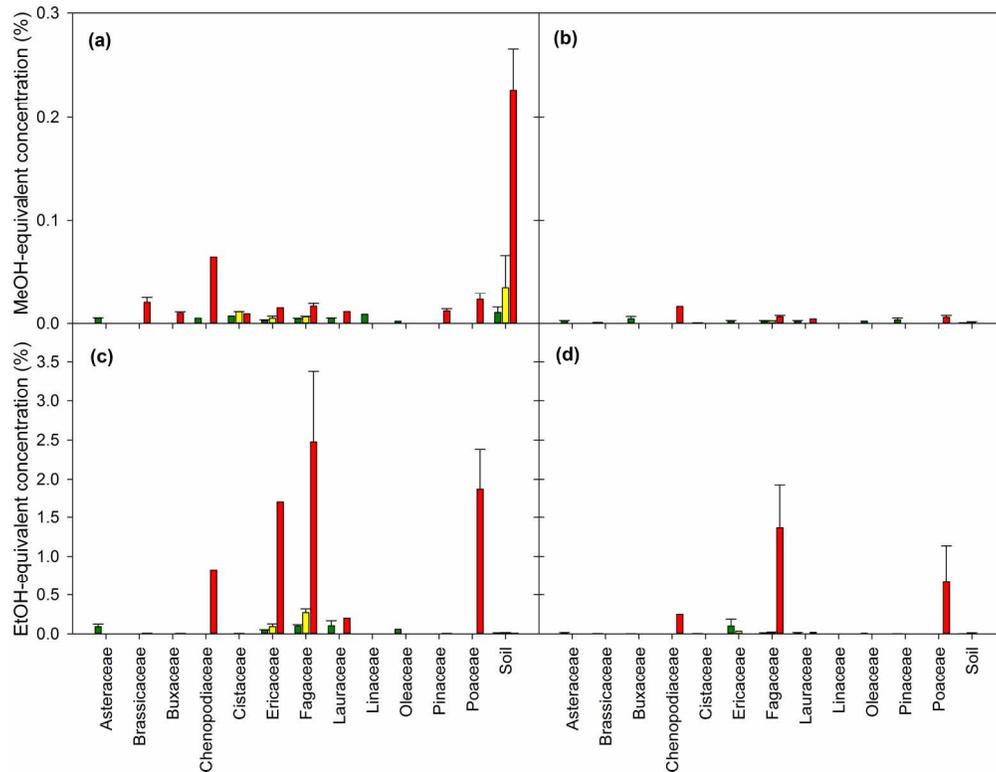


Fig. 4 Estimated equivalent concentrations (%) of methanol (MeOH, upper panels) and ethanol (EtOH, lower panels) by plant family, determined by fitting the spectral information provided by the Chemcorrect™ (respectively, 'ORGANIC_MEOH_AMPL' and 'ORGANIC_BASE' columns in the raw output files) against known values of MeOH and EtOH in the alcohol-water mixtures. Within each family, three coloured bars indicate the flagging categories of ChemCorrect™. Left and right panels show raw sample concentrations and concentrations after MCM pre-treatment, respectively.
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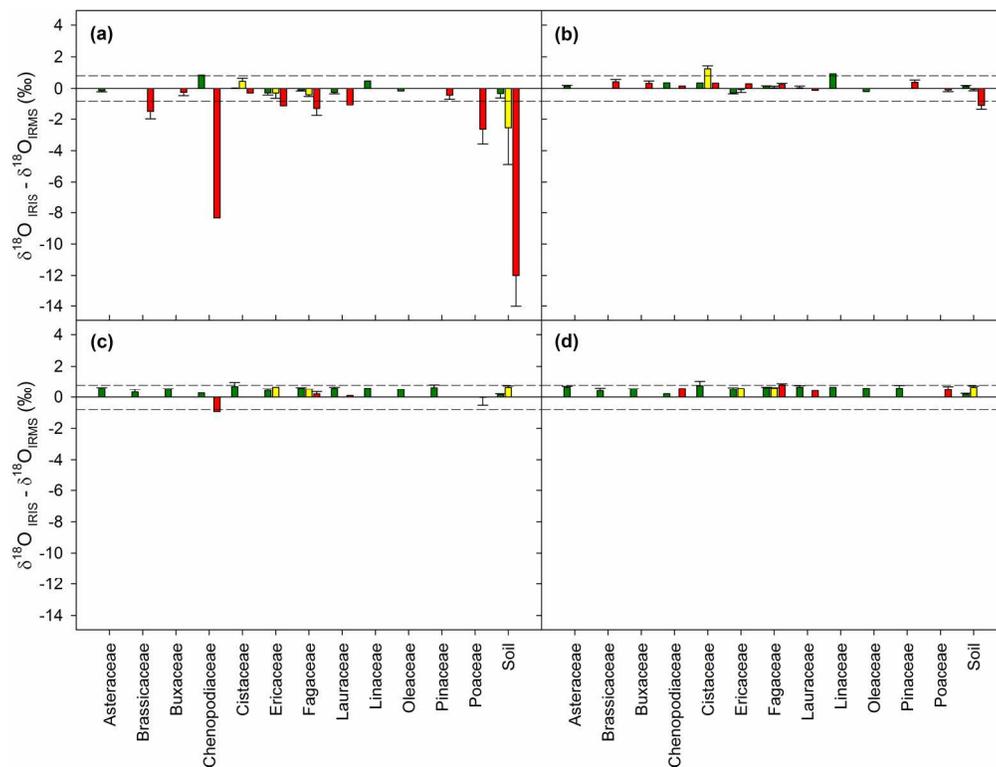


Fig. 5 Differences in $\delta^{18}\text{O}$ values between IRIS and IRMS by plant family. IRIS uncorrected (a), IRIS post-processed (b), IRIS plus MCM (c) and IRIS plus MCM plus post-processing (d). Error bars are standard errors. Dashed lines identify the maximum accepted bias (MAB) established by the International Agency of Atomic Energy. Colour codes represent the flagging categories of ChemCorrect™.

195x148mm (300 x 300 DPI)

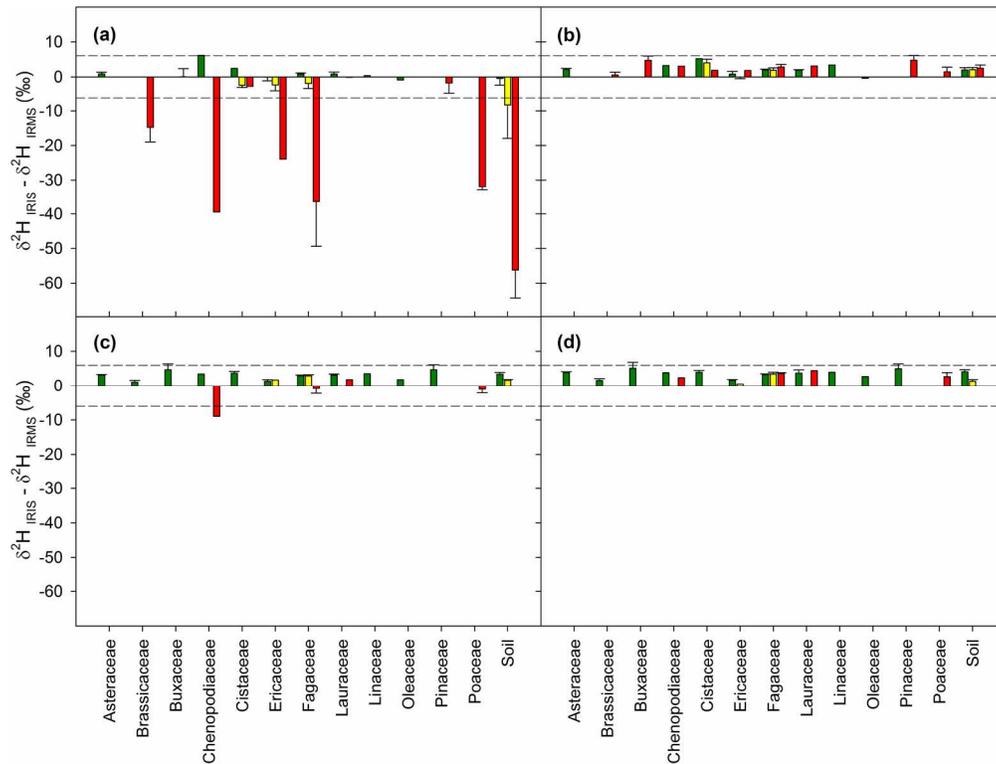


Fig. 6 Differences in $\delta^2\text{H}$ values between IRIS and IRMS by plant family. IRIS uncorrected (a), IRIS post-processed (b), IRIS plus MCM (c) and IRIS plus MCM plus post-processing (d). Error bars are standard errors. Dashed lines identify the maximum accepted bias (MAB) established by the International Agency of Atomic Energy. Colour codes represent the flagging categories of ChemCorrect™.

195x148mm (300 x 300 DPI)