Article:

1 **A comparison of methods for the extraction of dissolved organic matter**

2 **from freshwaters**

3 *Catherine S. Moody*

4 water@leeds, School of Geography, University of Leeds, UK, c.s.moody@leeds.ac.uk

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6 **Highlights**

7 • Methods of extracting dissolved organic matter from freshwaters vary in efficiency

8 • Dissolved organic matter was extracted from freshwaters in the UK

9 • Several methods were evaluated, and the time, cost and results of each assessed

10 • Rotary evaporation, freeze-drying and dry-down gave the most consistent results

11

12 **Graphical Abstract**
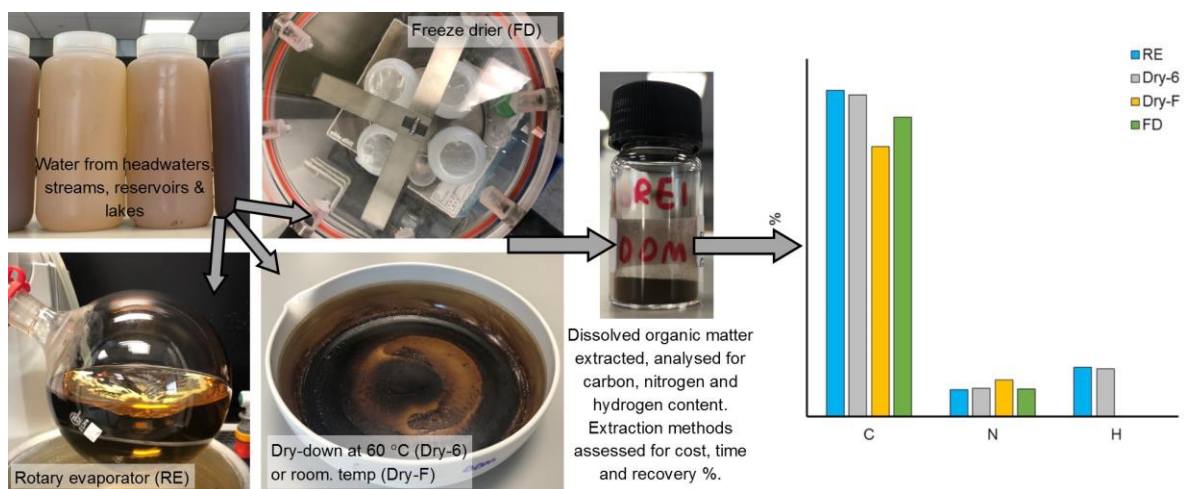
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15 **Abstract**

16 Studies of dissolved organic matter (DOM) composition have used several different methods

17 to concentrate and extract the DOM from fresh water, but the impact of these methods on the

18 composition of the DOM is relatively unknown, as very few studies use more than one

19 method to compare results. The aim of this study was to use several methods, frequently used

20   in the scientific literature, to concentrate and extract DOM from fresh water and compare the

21   elemental and functional group composition of the extracted DOM. In addition, the cost, in

22   terms of money, resources and time, were assessed for each method. The results showed that

23   the elemental and functional group composition of the extracted DOM varied between

24   methods significantly. The methods that yielded the most similar and reproducible DOM

25   results were rotary evaporation, dry-down at 60°C and freeze-drying. Although each of these

26   methods required a relatively expensive piece of laboratory equipment, this was a 'one-off'

27   cost, and consumables and time per sample were relatively low. This study highlights the

28   dangers of comparing DOM data from different studies when the DOM has been extracted

29   via different methods. In future, it is recommended that studies of DOM composition report

30   their methods of extraction clearly and consistently, ideally using one (or more) of the

31   methods showing reliable results here.

32   KEYWORDS: elemental analysis, rotary evaporation, freeze drying, reverse osmosis,

33   dialysis, dry-down

34

1. Introduction

36   Dissolved organic matter (DOM) is found in natural waters around the world, and plays an

37   important role in the transport of nutrients, energy and carbon from terrestrial ecosystems to

38   the atmosphere, aquatic and marine systems. Terrestrial inputs of carbon to inland waters are

39   estimated to be 5.1 Pg C year^{-1} (Drake et al 2018), and processing of DOM in lakes and rivers

40   releases 2.1 Pg C year^{-1} to the atmosphere as CO_2 (Raymond et al 2013). The composition of

41   DOM controls the reactivity, and therefore the proportion of the matter that degrades and

42   releases carbon dioxide (CO_2), or travels to the ocean (Bowen et al 2020). In order to better

43   understand the link between DOM in fresh water and other factors such as degradability,

44   catchment characteristics or water treatment efficiency, the DOM composition needs to be

45   known. Water companies in the UK and Northern Europe need to know how the composition

46   of DOM in their source waters impacts on the efficiency of drinking water treatment and the

47   potential for the formation of harmful disinfection by-products (such as trihalomethanes;

48   Kothawala et al 2017; Valdivia-Garcia et al 2019).

49   The DOM composition presented in existing literature on fresh waters may not be the 'true'

50   composition of the 'whole' sample, as the composition may be altered by the method, and

51   studies can only analyse the extractable fraction (Song et al 2018). With increased use of

52   advanced molecular characterisation methods (e.g. Fourier transform ion cyclotron resonance

53   mass spectrometry (FTICR-MS; Matilainen et al 2011)), it is important to be assured that the

54   organic matter being characterised is not just a by-product of the concentration method or

55   extraction method used.

56   Some methods attempt to classify and quantify the DOM 'in-situ' - while it is in solution in

57   natural water (Table 1). These methods include analysing its absorbance spectrum in the UV

58   and visible wavelengths (e.g. Dobbs et al 1972; Peacock et al 2014), or using excitation

59   emission fluorescence spectroscopy (e.g. Smart et al 1976; Chen et al 2003). Such approaches

60   rely on 'proxy' measurements to characterise the coloured components of DOM - the organic

61   matter is classified as 'humic-like', 'fulvic-like' or 'more aromatic' instead of quantifying the

62   composition directly. Classifying and quantifying the DOM 'in-situ' is generally simple,

63   relatively quick and inexpensive, and valuable information can be gathered, but depending on

64   the wavelengths used, they are limited to the coloured portion of DOM (CDOM), and a

65   proportion of DOM is 'optically invisible' to these techniques (Pereira et al 2014). The

66   results are also influenced by other factors of the water chemistry, such as pH and ionic

67   strength (Matilainen et al 2011). Both absorbance and fluorescence measurements were found

68   to be sensitive to a range of environmental conditions (such as pH, ionic strength and metal

69   ions), however this is likely related to the environmental conditions impacting the DOM

70   structure, and the subsequent measured absorbance and fluorescence spectra (Spencer et al

71   2007).

72   In order to analyse and understand DOM more thoroughly, the DOM can be extracted from

73   natural water, and analysed as a solid, or re-dissolved in higher concentrations than would

74   naturally occur (e.g. Søndergaard et al 2003; Lv et al 2016; Whitty et al 2019). Studies often

75   acknowledge the limitations of their extraction methods, and consequently refer to the DOM

76   extracted fraction of material as a proportion of the whole, total DOM. For example, Dittmar

77   et al (2008) refer to the DOM they extracted from seawater as "SPE-DOM". Very few studies

78   have used more than one method to extract the DOM, and so it is unknown how much the

79   method of extraction impacts the DOM composition analysed in the study (Minor et al 2014;

80   Tfaily et al 2012). Some researchers have used methods to concentrate the DOM before

81   extraction, such as Koprivnjak et al (2009), who used reverse osmosis and electrodialysis,

82   and Lankes et al (2008) who used rotary evaporation followed by freeze drying. Matilainen et

83   al (2011) carried out a review into the methods for characterising natural organic matter

84   (NOM – includes particulate and colloidal organic matter, not just the dissolved fraction),

85   including assessing the methods for concentrating and extracting NOM from water. Their

86   study focussed on methods used to extract a targeted fraction of the NOM, based on features

87   such as molecular size and polarity, and discussed the pros and cons of each method. By

88   targeting certain polarities and molecular sizes, the study acknowledged the methods included

89   were not analysing the total portion of NOM, and so not reflecting 'total' NOM (Matilainen

90   et al 2011). Whitty et al (2019) used [1]H-Nuclear Magnetic Resonance spectroscopy (NMR) to

91   compare DOM extracted by freeze-drying with 'whole water', and discussed the pros and

92   cons of other extraction methods. Despite these studies showing differences between NOM

93   and DOM fractions extracted by different methods, there is very little evidence showing how

94 the composition of the whole DOM composition is impacted by the extraction method. For

95 the purposes and focus of this study, organic matter smaller than 0.7 µm is classed as DOM.

96 A literature search was carried out to identify the most frequently used extraction and

97 concentration methods (Table 1). Each method identified in the literature search was entered

98 into Google Scholar (March 2020), along with "dissolved organic matter", "DOM", "water"

99 and "surface OR inland OR fresh", and the number of results used as a proxy to reflect how

100 popular/frequently used each method is. The most frequently used methods were found to be

101 rotary evaporation, freeze drying, dry-down, solid phase extraction, reverse osmosis and

102 dialysis. This is by no means a comprehensive study of all extraction methods, but includes

103 the most common methods for extracting DOM, regardless of molecular size and polarity,

104 from inland fresh waters. Resins, such as XAD-8, were not included in this study, as the

105 literature search revealed several uncertainties, questions and criticisms of the reliability and

106 extraction biases of this method, especially in regard to potential contamination from resin-

107 bleeding, and chemical alteration of samples (e.g. Kitis et al 2001; Sleighter et al 2009).

108 XAD-8 resins and the conditions needed to use them to extract DOM (such as pH changes)

109 have been showed affect the characteristics and reactivity of the DOM fractions (Song et al

110 2009).

111

112 Table 1. The methods and techniques used to concentrate, extract and analyse DOM

113 composition, and pros and cons of each method. Literature references listed include an early

114 study using the technique and a recent example of use in a study on DOM composition. The

115 number of results in a Google Scholar search (March 2020) containing the method and

116 "dissolved organic matter", "DOM", "water" and "surface OR inland OR fresh", are shown

117 in the final column.

Method	Pros	Cons	References	Google Scholar N
Absorbance	Easy and quick	Some DOM is colourless	Dobbs et al 1972 Peacock et al 2014	21300
Fluorescence	Proxy composition information Relatively quick	Some DOM does not fluoresce	Smart et al 1976 Chen et al 2003	21000
Rotary evaporation (RE)	Increased surface area for vaporization of the solution	Uses heat, which could alter the water chemistry	Craig et al 1950 Dean et al 2019	444
Solid phase extraction (SPE)	Relatively inexpensive, reliable	Extracted fraction depends on column, polarity, solvent	Thurman and Mills 1998 Lv et al 2016	3850
Dry-down (DRY)	Low-tech	Uses heat, which could alter the water chemistry At room temperature, microbial growth	Veitch 1904 Worrall et al 2018	101
Reverse osmosis (RO)	Concentrates DOM relatively quickly	Recovery percentage affected by pH of water	Hauck and Sourirajan 1969 Green et al 2015	3360
Freeze-dry (FD)	Results in a solid DOM without any other processing	Expensive equipment	Geiger and Cataldo 1969 Lankes et al 2008	127
Dialysis (DIA)	Resulting DOM contains fewer 'impurities' such as salts	Membrane size may exclude smaller DOM molecules	Tan 1977 Aristilde et al 2017	2380

118

119   The aim of this study was to compare different extraction methods used in the literature to

120   quantify the impact on the elemental composition of the resulting DOM. While no extraction

121   method can claim to be perfectly replicating the 'natural' composition of DOM as it would be

122   in the water, this study aims to find the method that causes the least disruption to the

123   composition, is economical (in terms of cost, resources and time) and is replicable and

124   reliable.

125

126   **2. Method**

127   The DOM extracted from natural water using nine different methods was assessed. The time

128   and costs of each method were recorded, and elemental and functional group composition of

129   the DOM was analysed.

130

131   **2.1. Sample collection**

132   Water samples of between 5 and 20 L were taken from 14 fresh water bodies across the UK,

133   including natural lakes, reservoirs and upland streams to ensure a variety of dissolved organic

134   carbon (DOC) concentrations and DOM compositions (Table S1). The sites were located

135   between 53.4 and 59.3 °N, and -3.9 and -0.7 °E. Samples of water from each water body were

136   filtered (DI-rinsed 0.45 µm syringe filters) and analysed for DOC concentrations (Analytik

137   Jena Multi NC2100 combustion analyser). The DOC concentrations ranged from 4.7 to 44.4

138   mg L^{-1}.

139

140   **2.2. DOM extraction methods**

141   All water for DOM extraction was filtered through 0.7 µm glass microfiber filters (VWR).

142   This size of filter was used so as to include the colloidal fraction of organic matter, but

143   exclude particulates. The filtered water was then split and subject to various methods to

144   extract the DOM. The time taken, resources needed and mass of DOM collected via each

145   method were recorded. Some methods were combined as the primary method concentrated

146   rather than fully extracted the DOM and therefore did not result in a solid DOM sample.

147

148   <u>2.2.1. Rotary Evaporation (RE)</u>

149   One litre of filtered water was poured into an evaporating flask. The water bath temperature

150   was 60°C, and cold tap water was used to cool the condenser, which was in a vacuum. The

151   evaporating flask was topped up with filtered water as the 'clean' water in the sample

152   evaporated and condensed in the receiving flask (Cranwell et al 2017). At most, 5 L of

153   sample water was used. 'Clean' water from the receiving flask was routinely collected,

154   filtered and analysed for DOC concentration. This clean water had a mean DOC

155   concentration of 0.23 mg L^{-1} (range 0-0.96 mg L^{-1}; n = 25). A t-test showed there was

156   significantly less DOC in the clean water than the initial water samples ($p < 0.001$). At most,

157   less than 1 mg L^{-1} of DOC was discarded in the clean water.

158   The concentrated water remaining in the evaporating flask (less than 500 mL) was poured

159   into a 1.1 L evaporating dish which was then put in an oven at 60 °C until the liquid had

160   evaporated. The residue was then collected. This method was used on water from all 14 water

161   bodies.

162   In order to analyse the reproducibility of the rotary evaporation method, water samples from

163   a further 15 UK fresh waters were split into two and rotary evaporated, resulting in 15 pairs

164   of DOM samples (n = 30). These samples were not included in the 'time, cost and recovery

165   analysis', but were subject to elemental analysis (as in 2.3.).

166

167   2.2.2. Solid Phase Extraction (SPE)

168   Known volumes of up to 1 L of filtered water were poured through Bond Elut PPL 500 mg, 6

169   mL cartridges (Agilent) conditioned with 6 mL 100% methanol followed by 6 mL deionized

170   water. This cartridge was chosen based on a search of literature using solid phase extraction,

171   and found to be the type recommended for DOM extraction from natural water (Li et al

172   2019). The sample water was forced through the cartridge using a hand pump to create a

173   small pressure gradient. The cartridge was then washed in 6 mL deionized water, air dried

174   using the hand pump to pull air through cartridge. To elute the DOM sample, 12 mL of 100%

175   methanol was used, which was then evaporated off at room temperature and the residue

176   collected. This method was used on water from three sites.

177

178   2.2.3. Dry down (DRY-6, DRY-F)

179   The filtered water was poured into a 1.1 L evaporating dish and then kept either in a fume

180   hood at room temperature (DRY-F) or in an oven at 60 °C (DRY-6) until all liquid had

181   evaporated. The evaporating dish was chosen so as to have a large surface area to volume

182   ratio. The evaporating dish was topped up routinely, until approximately 5 L of water had

183   evaporated. The residue was then collected. Dry down in the oven (DRY-6) was used on

184   water from 13 sites, and dry down in the fume hood (DRY-F) was used on water from six

185   sites.

186

187   2.2.4. Reverse Osmosis (RO) followed by rotary evaporation (RE) or dry down (DRY-F)

188   Reverse osmosis removes ions and large particles from water under pressure, with the aim of

189   providing cleaner water. Approximately 10 L of the 0.7 μm filtered water was pumped

190   through a customised reverse osmosis unit. The unit contained a 5 μm polypropylene filter, a

191   1 μm polypropylene filter, and a carbon filter. The whole unit and filters were washed in

192   deionised water before each sample was pumped through. The 'dirty' water was fed back into

193   the unit and the 'clean' water collected separately, until the majority of the water was 'clean'.

194   Samples of this 'clean' water were collected, filtered and analysed for DOC concentration.

195   The analysis of the clean water showed that it contained an average of 8 mg L^{-1} DOC. This

196   was almost as high as the DOC concentration in some of the initial water samples, showing

197   that a lot of the DOC in the original water was getting through the filters into the clean water

198   fraction. The clean water fraction was discarded, and the final dirty water fraction, containing

199   less DOM, was collected. The carbon filters were rinsed in DI water, and this was added to

200   the dirty water (as it could have retained carbon molecules). Due to the high concentration of

201   DOC in the clean water, this method was not continued.

202   Once the volume of 'dirty' water was approx. 2 L, this was collected, then split, with 1 L

203   further evaporated in the rotary evaporator (RO-RE; as in 2.2.1.) and 1 L evaporating to

204   dryness in an evaporating dish at room temperature (RO-DF; as in 2.2.3.). Both reverse

205   osmosis methods were used on water from two sites.

206

207   <u>2.2.5. Freeze Drier (FD)</u>

208   A known volume of filtered water was placed in the freeze drier at -50 °C until the water had

209   sublimated, and the remaining solid material was collected. This method was used on water

210   from five sites.

211

212   <u>2.2.6. Dialysis (DIA) followed by FD or DRY-F</u>

213   A total of 200 mL of filtered sample water was poured into regenerated natural cellulose

214   membrane dialysis tubing (10 kDa MWCO (molecular-weight cut-off), 29 mm diameter,

215   Spectrum Labs), clipped at the bottom and top. The dialysis tubing was then put into a beaker

216   of deionised water. The deionised water was replaced regularly. The water was split into two

217   100 mL volumes, one of which was put in the freeze drier (DIA-FD; as in 2.2.5.), while one

218   was left to evaporate at room temperature (DIA-FH; as in 2.2.3.). Both dialysis methods were

219   only used on water from one site.

220

221   **2.3. DOM analysis**

222   The extracted DOM was weighed, then analysed by elemental analysis to measure the content

223   of carbon, nitrogen and hydrogen in the samples. The samples were treated with hydrochloric

224   acid to remove inorganic carbonates, then analysed again for the organic carbon proportion.

225   A sub-set of samples (n = 23) were also analysed by solid state ^{13}C nuclear magnetic

226   resonance (NMR) to look for differences in functional group distribution. The method of

227   NMR used means that directly quantifying the results was not possible; however, they can be

228   compared to each other, and expressed relative to the amount of total C in the DOM sample

229   (Hockaday et al 2009). The 23 samples analysed by NMR included DOM extracted from nine

230   sites (sites 1-9), by six different methods (both dry down methods, freeze drying, rotary

231   evaporation, and both reverse osmosis methods).

232   Due to various time, equipment and water constraints, not all extraction methods were used

233   on water from all sites. One method, rotary evaporation (RE), was carried out on water from

234   all sites (n = 14), so the composition data were analysed relative to the RE extracted DOM

235   sample. This does not mean that the composition of the RE extracted DOM was presumed to

236   be the 'true' DOM composition, only that this method was applied to water from all 14 sites.

237

238   **2.4. Time, cost and recovery**

239   In order to evaluate each method, the following were recorded, and ranked from 'best' to

240   'worst':

241   • The time taken from the start of sample processing to collecting the final DOM sample,

242   and the **rate of processing** (litres per hour). Methods were considered 'better' if they had

243   a relatively fast processing rate, or took a shorter amount of total time.

244   • The **recovery percentage**, relative to the DOC concentration of the original water sample.

245   Recovery percentages of over 100% were possible, as the water used for DOM extraction

246   was filtered through a larger filter size than the water analysed for DOC concentration (0.7

247   vs 0.45 µm filter) in order to include colloidal organic matter. Ideally, the carbon content

248   of the DOM would be similar to the carbon concentration in the original water, resulting in

249   a recovery percentage close to 100%. Rank scores were allocated based on how far from

250   100% the mean values fell. The recovery percentage for each sample was compared to

251   various water chemistry variables, to investigate which properties of the water or DOM

252   influenced the recovery percentage of the DRY-6, DRY-F, FD and RE extraction methods

253   (where n > 2). The DOM samples extracted by RE and DRY-6 methods (where n > 10)

254   were also analysed grouped by water body type (e.g. headwaters and streams). Variables

255   used to calculate the recovery percentage (DOC concentration and carbon content of the

256   DOM) were not included in this analysis.

257   • The approximate **cost of consumables per sample**. Lower costs per sample were

258   considered 'better'.

259   • If the method required any larger laboratory equipment (lab oven, rotary evaporator,

260   freeze drier), these were not included in the cost per sample, but were considered when

261   ranking the total cost per method. The '**large equipment score**' was the cost of the large

262   equipment divided by 1000, to give a number between 0 and 10.

263   Certain costs were not included, such as electricity and tap water, but these were reflected in

264   the total time taken. Costs of collection and analysis of samples was not included as this will

265   be the same for all samples. The three criteria (rate of processing, recovery percentage and

266 cost per sample) were ranked, and added to the large equipment score. Each method was

267 allocated a score based on the sum of these.

268

269 **3. Results**

270 A total of 47 DOM samples were successfully extracted by all methods (Tables 2 and 3). The

271 additional 15 pairs of rotary evaporated samples were analysed separately (n = 30).

272

273 Table 2. The mean average total carbon, nitrogen and hydrogen, and organic carbon, relative

274 to the average content of the RE DOM. The closer the value to 1, the more similar the DOM

275 composition is to the RE DOM composition. Values in brackets are the standard errors.

Method	N	Total C	Total N	Total H	Organic C
dry down at 60 °C	13	0.99	1.07	0.97	0.95
(DRY-6)		(0.02)	(0.03)	(0.02)	(0.03)
dry down at room. temp.	6	0.83	1.51	1.08	0.81
(DRY-F)		(0.04)	(0.21)		(0.06)
reverse osmosis-rotary evaporation	2	0.46	0.14	0.56	0.17
(RO-RE)		(0.03)	(0.08)	(0.03)	(0.13)
reverse osmosis-dry down at room. temp.	2	0.49	0.23	0.51	0.12
(RO-DF)		(0.07)	(0.12)	(0.06)	(0.09)
freeze drier	5	1.05	1.02	-	1.04
(FD)		(0.09)	(0.02)		(0.03)
dialysis-freeze drier	1	2.06	1.55	-	-
(DIAFD)					
dialysis-dry down at room. temp.	1	1.04	2.59	-	-
(DIAFH)					

276

3.1. Elemental and functional group composition

The results show that the most significant differences in DOM compositions were between the sites, rather than the method of extraction, and so all further analysis of elemental data was carried out on the composition relative to the 14 rotary evaporated (RE) DOM composition for each site (Table 2). The RE DOM composition was used as this method was carried out on water from all sites. The closer the value to 1, the more similar the extracted DOM composition is to the RE DOM composition. The solid phase extraction (SPE) method resulted in DOM samples that were too small to analyse further, so it was not continued.

The average elemental composition of the dry down at 60 °C (**DRY-6**) DOM was between 0.95 and 1.07 of the RE DOM values. There was not enough H data for the DOM extracted by freeze drier (**FD)**, but for total and organic C, and total N, the average results across sites were 1.05, 1.04 and 1.02 of the RE DOM values, respectively. A one-way ANOVA on extraction method (RE, DRY-6 and FD) showed no significant differences ($p > 0.05$) in the raw composition data (total C, N, H, and organic C) between the three methods. These data showed that despite the differences in methods, involving changing the temperature and pressure of the samples, the DOM extracted was similar in elemental composition.

The reverse osmosis (**RO-RE** and **RO-DF)**, dry down at room temperature (**DRY-F)** and dialysis (**DIAFH** and **DIAFD**) methods resulted in samples with very different composition data, varying between 0.12 and 2.59 of the RE data. It was not surprising that the dialysis method DOM was different to the other methods, as the size of membrane used allows smaller molecules to pass through it, retaining larger molecules. This resulted in the loss of very low molecular weight DOM, resulting in different DOM composition. From the DOC concentration of the 'clean' fraction of the water disposed of by the reverse osmosis (average

302   8 mg L^{-1}), it was clearly not extracting all of the DOM in the water, and so the elemental

303   analysis of the resulting DOM would be different to that extracted by other methods. The

304   DRY-F method took an average of 392 hours per sample (more than 16 days) to evaporate

305   less than 2 L of water. During this time, the water was warm and light; any microbes smaller

306   than 0.7 µm would have remained after filtration and could have degraded the DOM,

307   releasing CO_2. This would have led to a preferential loss of the more reactive carbon,

308   changing the DOM composition and DOC concentration, as reflected in the lower recovery of

309   DOM (69%).

310   The additional 15 pairs of rotary evaporated samples had incredibly similar results – paired t-

311   tests showed there were no significant differences in the total C (p = 0.46), N (p = 0.34), or H

312   (p = 0.41) between the replicates (n = 30).

313   The ^{13}C-NMR was carried out on 23 samples from nine of the 14 field sites (Figure 1).

314   Similar samples will have similar spectra – the lines will be close together and follow a

315   similar pattern. If the samples have different functional group compositions, the lines will be

316   different.

317   As with the elemental composition data, these data showed similar results for the **RE** and

318   **DRY-6** methods, and more variable results for the **RO-RE** and **RO-DF** samples. In the water

319   from Site 1, the RO-RE and RO-DF methods have very low intensities at the majority of

320   ppms, whereas the DRY-6 and RE samples have very similar spectra. Peaks in the lower

321   range ppms (below 100) reflect functional groups such as C-alkyls, N- alkyls and O-alkyls,

322   and these seem to be present in the DRY-6 and RE samples, but much lower or even missing

323   from the RO-DF and RO-RE samples.

324   The RE and DRY-6 methods also had very similar results in water from study sites 2, 3, 6, 7,

325   8 and 9. The DOM from Site 4 are the only samples where the DRY-6 and RE samples have

326   different intensity results, however the majority of the peaks are at the same ppm locations.

327   The DOM from water from Site 5 was extracted by FD and RE, and both spectra show

328   similar patterns, but much lower intensities than in samples from other sites. The four

329   samples from Site 9 show similar spectra to Site 1, although the RO-DF sample has the

330   highest intensities and RO-RE has the lowest – missing several of the peaks present in the

331   other samples.

332

333   Figure 1. The NMR spectra for each sample (n = 23, from 9 sites). The 'intensity' (y-axis) is

334   relative to the total C in each sample. Similar samples will have similar spectra – the lines

335   will be close together and follow a similar pattern. If the samples have different functional

336   group compositions, the lines will be different.

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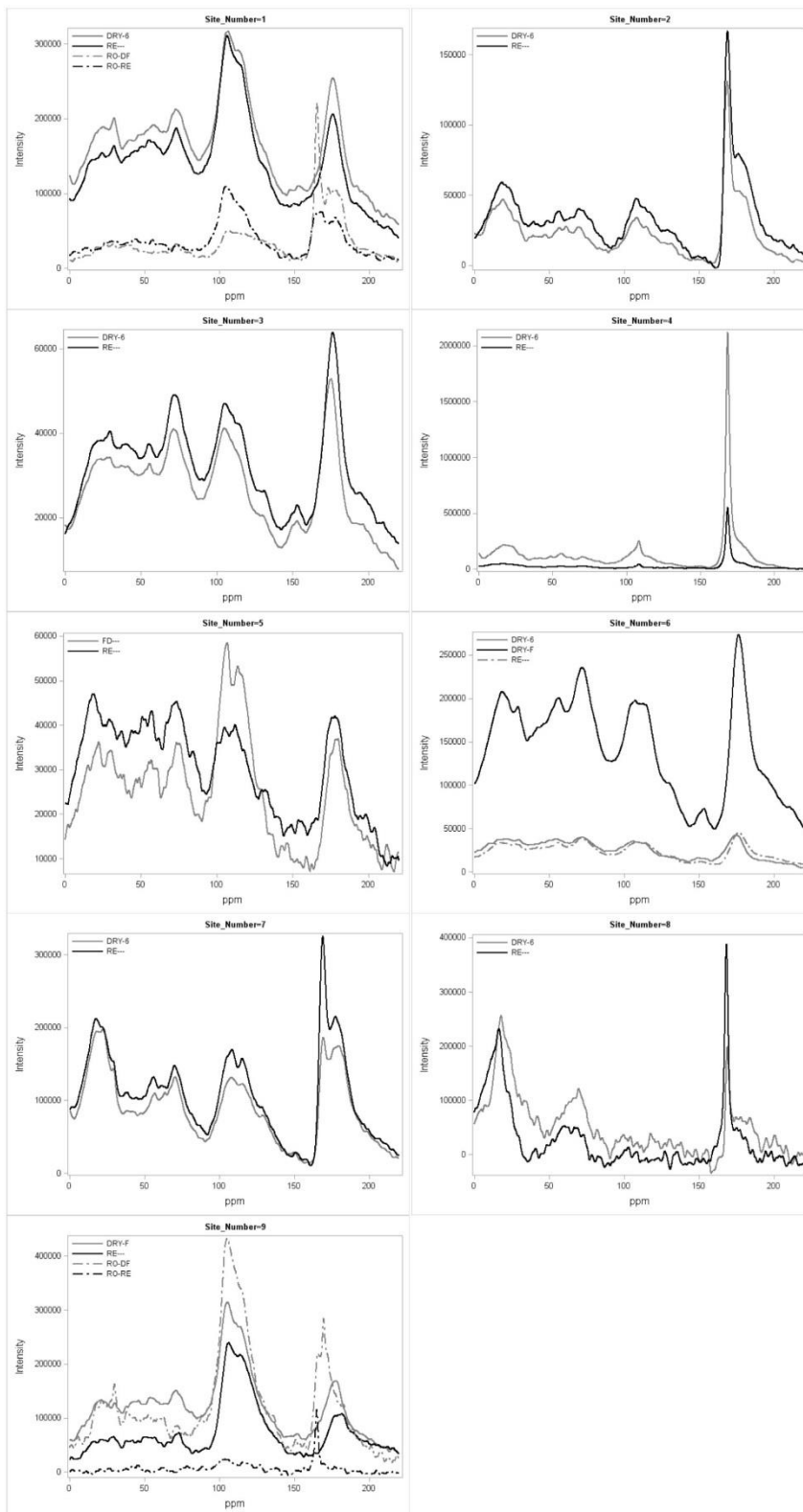
3.2. Time, cost and recovery

341 The results of this analysis show that the methods ranked as 'best' with the lowest overall

342 scores were the rotary evaporation and dry-down methods (Table 3). The **SPE** method

343 resulted in DOM samples that were too small to analyse further, so it was not continued. It

344 was also relatively expensive per sample. The resulting overall score was 17, and therefore it

345 was ranked fourth.

346

347 Table 3. The rate (mL hr-1), recovery percentage (± standard error) and cost per sample

348 (GBP and USD) for each method. The overall score is the sum of the ranks of rate, recovery

349 percentage and cost per sample, and large equipment score (lower number = better method).

350 The notation 'n.a.' = no additional equipment and/or costs. Rank and score values are in

351 italics.

Method	Rate (mL hr^{-1}) and *rank*	Recovery (% ± SE) and *rank*	Cost per sample (£, $) and *rank*	Large equipment cost (£, $) and *score*	Overall score and *rank*
RE	897.44	100 (11)	9.43, 12.39	2000, 2630	10
	1	*1*	*6*	*2*	*1*
DRY-6	39.94	93 (8)	2.21, 2.90	2000, 2630	10.5
	2	*2*	*2.5*	*2*	*2*
DRY-F	4.59	69 (10)	2.21, 2.90	n.a.	12.5
	6	*4*	*2.5*	*0*	*3*
SPE	503.33	-	102.67, 134.91	n.a.	17
	2	*8*	*7*	*0*	*4*
RO-RE	100.40	158 (87)	384.50, 505.25	2000, 2630	18.5
	3	*5*	*8.5*	*2*	*5*
FD	5.36	89 (6)	n.a.	10000, 13140	19
	5	*3*	*1*	*10*	*6*
DIA-FH	0.46	37	8.79, 11.55	n.a.	20.5

	9	*7*	*4.5*	*0*	*7*
RO-DF	2.92	159 (75)	384.50, 505.25	n.a.	21.5
	7	*6*	*8.5*	*0*	*8*
DIA-FD	1.39	237	8.79, 11.55	10000, 13140	31.5
	8	*9*	*4.5*	*10*	*9*

The **RE method** had a low cost per sample and the recovery percentage of DOM was high (100 ± 11%). Water samples with a low starting DOC concentration (less than 10 mg L^{-1}) had a much more variable recovery percentage (range 36 to 183%), whereas water samples with higher starting DOC concentration (above 10 mg L^{-1}) had a lower recovery percentage range (64 to 147%). The recovery percentage was positively correlated with the pH of the source water ($p = 0.05$, $R^2 = 0.28$, $n = 14$); DOM samples with lower recovery percentages were from water sources with lower pH values. The relationships between pH and recovery percentage were stronger when the DOM samples were grouped according to the type of water body sampled. The pH of the source water explained the majority of the variation in the DOM recovery percentages from headwaters and streams samples ($p < 0.01$, $R^2 = 0.97$, $n = 6$). The method overall score was 10; this was the lowest overall score of all the methods and so this method was ranked first.

DRY-6 and **DRY-F** were relatively simple, requiring the least equipment of all methods tested; therefore, the cost was very low per sample. The recovery rate of DRY-6 was 93%, whereas the recovery rate from DRY-F was 69%. The recovery percentage of DOM extracted by the DRY-F method was negatively correlated with the pH of the source water ($p = 0.04$, $R^2 = 0.71$, $n = 6$); DOM samples with lower recovery percentages were from water sources with higher pH values. There was no significant correlation between the DOM recovery percentage and water pH in samples collected by the DRY-6 method ($p = 0.47$, $n = 13$). When grouped by water body type, the samples from headwaters and streams had a

373 significant negative relationship ($p < 0.01$, $R^2 = 0.89$, $n = 6$). The recovery percentage of

374 DOM extracted by the DRY-6 method was weakly positively correlated with the nutrient

375 concentrations (nitrate and nitrite, total nitrogen (N) and total phosphorous(P)) of the source

376 water; however the majority of values were very close to or at the detection limit of the

377 analysers. It is possible that DOM samples with lower recovery percentages were from water

378 sources with lower nitrate, nitrite, total N and total P. Their respective overall scores were

379 10.5 (DRY-6) and 12.5 (DRY-F), making them ranked second and third.

380 The **FD method** was also very simple; however, the freeze-drier was the most expensive

381 single piece of equipment used. This was the only method that directly resulted in solid DOM

382 samples without having to evaporate off the remaining water (either at room temperature or at

383 60 °C in an oven). The recovery rate of this method was 89%, with the smallest range of any

384 method tested in the experiment (74 to 112%). There were positive correlations between the

385 absorbance at eight UV and visible light wavelengths (665, 470, 465, 436, 400, 360, 265 and

386 254 nm) and the recovery percentage of the five DOM samples extracted by FD. Water

387 samples with higher absorbance values had higher DOM recovery percentages. The recovery

388 percentage was weakly positively correlated with the nutrient concentrations (nitrate and total

389 P) of the source water; however the majority of values were very close to or at the detection

390 limit of the analysers. It is possible that DOM samples with lower recovery percentages were

391 from water sources with lower nitrate and total P. The FD method overall score was 19, and it

392 was ranked sixth, increased by the cost of the large equipment needed.

393 **RO-RE** and **RO-DF** had very similar results to each other; however, the RO equipment was

394 relatively expensive per sample. The RO methods were carried out on water from two sites:

395 the site with lower DOC concentration (8.6 mg L^{-1}) resulted in very high recovery

396 percentages (234% RO-DF; 245% RO-RE), whereas the site with higher DOC (21.1 mg L^{-1})

397   resulted in lower recovery percentage (84% RO-DF; 71% RO-RE). Their respective overall

398   scores were 18 (RO-RE) and 22 (RO-DF), ranked fifth and eighth overall.

399   The **DIA-FD** and **DIA-FH** methods were limited by the volume of water that could be

400   dialysed at each time, resulting in small DOM samples. The dialysis tubing is relatively

401   expensive and to extract a larger DOM sample would have increased the cost of this method.

402   The water sample that was subject to dialysis had a starting DOC concentration of 44.4 mg L^-

403   ^{1} (the highest of all the samples used in this study), but the DIA-FH had one of the lowest

404   (and therefore worst) recovery percentages (37%), showing a lot of the carbon measured in

405   the DOC concentration was not extracted and present in the DOM. The overall scores for the

406   two methods using dialysis were 20.5 (DIA-FH) and 31.5 (DIA-FD), ranked seventh and

407   ninth overall.

408

409   **4. Discussion**

410   The DOM extracted from natural water using nine commonly used methods was assessed.

411   The time and costs of each method were recorded, and elemental and functional group

412   composition of the DOM was analysed. The results showed that the most reliable and similar

413   composition results were obtained from the rotary evaporator (RE), freeze dryer (FD) and dry

414   down at 60 °C (DRY-6) methods (Table 2; Figure 1). The consistent DOM composition and

415   NMR results across these methods suggest they extracted a 'real' DOM composition, or that

416   all three methods altered the elemental and functional group composition in the same way.

417   The rotary evaporator, freeze dryer and dry down at 60 °C methods also had relatively fast

418   rates and low costs per sample (Table 3). The scoring system resulted in a higher score

419   (therefore a 'worse' method) for methods that included a freeze dryer, due to the large

420   equipment cost (~£10,000, Table 3). Calculating the overall score without the large

421   equipment cost, the lowest scores (and therefore 'best' methods) are RE, DRY-6 and FD

422   (scores are 8, 8.5 and 9), the same three methods as found to be the most reliable and

423   consistent in the elemental analysis. The high cost of the freeze drier is a one-off cost, and

424   could be offset by the practically zero consumable costs per sample.

425   Thacker et al (2005; 2008) used rotary evaporation to extract DOM from water. They used a

426   water bath at 45 °C, but the temperature of the evaporating sample was measured as 20 °C.

427   They were confident that the temperatures involved would not result in losses of DOM, and

428   report extracted recoveries of between 70 and 91% (2005 study) and 93 to 107% (2008

429   study). The slightly higher temperature water bath used in the current study (60 °C) would

430   likely result in a 35-40 °C evaporating temperature for the sample; the high recovery rates

431   indicate that there was hardly any loss of volatile carbon compounds at these temperatures.

432   The only difference between the RE and DRY-6 methods in this study was the vacuum in the

433   rotary evaporator; in both methods the water was heated to 60 °C. The elemental and

434   functional group compositions were most similar between these two methods.

435   The water chemistry of the source water influenced the recovery percentages. The pH of the

436   source water was found to impact the recovery percentage of the DOM samples extracted by

437   both RE and DRY-F, but not DRY-6 or FD. Higher water pH increases the solubility of

438   DOM; it is possible that this impacts the recovery percentage during rotary evaporation and

439   dry down. Higher absorbance values in water may lead to higher recovery percentages of

440   DOM extracted by freeze drying, and lower nutrients (especially nitrate and total

441   phosphorous) may lead to lower recovery percentages of DOM extracted by freeze drying

442   and dry down at 60 °C (although more work needs to be done to investigate this further).

443   High absorbance values in water often indicate high DOC concentrations, especially in

444   waters draining catchments dominated by peat soils (Wallage and Holden 2010), and so it is

445   likely that the correlation between absorbance and recovery percentage is controlled by high

446   DOC concentrations in samples with high absorbance values. In future studies, if DOM is to

447   be extracted using these methods from source waters with a wide range of pH and absorbance

448   values, then the recovery percentage will likely be affected.

449   Søndergaard et al (2003) used freeze-drying to extract DOM from estuary water and found a

450   97% recovery. They compared the freeze-dried DOM to 'fresh' and found no differences in

451   DOC bioavailability, suggesting the freeze-drying process did not alter the DOM

452   significantly. The freeze-dried DOM allowed them to artificially manipulate the DOM and

453   DOC concentration without adding water (and therefore changing the water chemistry of

454   their samples), another benefit of extraction. Whitty et al (2019) compared DOM extracted by

455   freeze-drying with 'whole water' from two water sources, and showed a 16% and 26% total

456   change in relative intensities across six integrated regions of ^{1}H-NMR spectra. The freeze-

457   dried samples lost oxygenated functional groups – these differences were attributed to

458   changes in the DOM composition during the freeze drying process, suggesting structural

459   changes do take place in the freeze-drying samples (Whitty et al 2019).

460   Kitis et al (2001) investigated the impact of reverse osmosis on DOM reactivity. They found

461   minimal loss of DOM (94-98% recovery) in water with DOC concentrations ranging from 2

462   to 25 mg L^{-1}, similar to this study. This shows that reverse osmosis can be used to recover

463   DOM, however the method used in Kitis et al (2001) was much more complicated, requiring

464   customised equipment, and therefore likely to be more costly in money and time than the

465   method used here.

466   The aim of this study was not to question the reliability of DOM composition data extracted

467   by different and varied methods used in past studies that have provided interesting and

468   valuable results, but to highlight the risks associated with comparing across studies. If the aim

469   of a new study is to compare DOM to previously published research, it is recommended that

470   the exact same method of DOM extraction be used. This also highlights the importance of

471   systematic and thorough reporting of the extraction methods used in studies of DOM

472 composition. In future studies of DOM extraction, especially from fresh waters, the methods

473 recommended are: rotary evaporation at 60 °C, freeze drying, and dry-down at 60 °C, as

474 these have provided the most reliable and consistent results.

475

476 **5. Conclusion**

477 DOM was shown to be highly variable in elemental and functional group composition using

478 samples from 14 water bodies across the UK. The results showed significant differences in

479 composition between DOM extracted via different, commonly used methods. The DOM

480 compositions were most consistent and reliable when extracted using the rotary evaporating,

481 freeze drying and dry-down at 60 °C methods. Using a rank and scoring system based on

482 time, cost, resources and recovery percentages, the ideal methods were identified as rotary

483 evaporation and dry-down at 60 °C. Freeze drying, despite having the greatest large-

484 equipment cost, is also a recommended method, as it had the lowest cost per sample and a

485 high recovery percentage.

486 The results show that composition data of DOM extracted by different methods are not

487 comparable. It is recommended that future studies of DOM composition use one of the

488 methods suggested, systematically report the method used and the recovery percentage, and if

489 necessary, extract DOM by more than one method for comparison. If comparing DOM

490 compositions from earlier literature, it is recommended to follow the method of extraction

491 used in the original work, to ensure any differences are not due to the extraction method.

492

503   **6. References**

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