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The interplay between total mercury, methylmercury and dissolved organic matter in fluvial systems: A latitudinal study across Europe

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Abstract

54 Large-scale studies are needed to identify the drivers of total mercury (THg) and
55 monomethyl-mercury (MeHg) concentrations in aquatic ecosystems. Studies attempting to
56 link dissolved organic matter (DOM) to levels of THg or MeHg are few and geographically
57 constrained. Additionally, stream and river systems have been understudied as compared to
58 lakes. Hence, the aim of the study was to examine the influence of DOM concentration and
59 composition, morphological descriptors, land uses and water chemistry on THg and MeHg
60 concentrations and the percentage of THg as MeHg (%MeHg) in 29 streams across Europe
61 spanning from 41°N to 64 °N. THg concentrations (0.06 – 2.78 ng L⁻¹) were highest in
62 streams characterized by DOM with a high terrestrial soil signature and low nutrient content.
63 MeHg concentrations (78 – 160 pg L⁻¹) varied non-systematically across systems.
64 Relationships between DOM bulk characteristics and THg and MeHg suggest that while soil
65 derived DOM inputs control THg concentrations, autochthonous DOM (aquatically produced)
66 and the availability of electron acceptors for Hg methylating microorganisms (e.g. sulfate)
67 drive %MeHg and potentially MeHg concentration. Overall, these results highlight the large
68 spatial variability in THg and MeHg concentrations at European scale, and underscore the
69 importance of DOM composition on Hg cycling in fluvial systems.

70

71 **1. INTRODUCTION**

72 Mercury (Hg) is a hazardous substance with the potential to produce significant
73 adverse neurological and other health effects in severely exposed humans, with particular
74 concerns on unborn children and infants (Mason et al., 2012). Anthropogenic emissions and
75 long-range transport of atmospheric-Hg have led to Hg concentrations above background
76 levels in surface reservoirs (atmosphere, oceans or terrestrial) on a global scale (Amos et al.,
77 2013). Aquatic food webs are particularly vulnerable to increases in Hg, since its methylated
78 form (monomethyl-mercury, MeHg) accumulates in organisms and biomagnifies in aquatic
79 food webs (Morel et al., 1998), even at low concentrations. As a consequence, millions of
80 people are readily exposed to harmful concentrations of MeHg via fish consumption (Mason
81 et al., 2012).

82 In order to prevent human exposure to Hg, a global legally binding agreement, the
83 Minamata Convention, was signed by 128 countries and entered into force in September
84 2017. Prior to the Minamata Convention, the European Water Framework Directive (WFD,
85 2008) classified Hg as one of 30 priority hazardous substances and set the maximum
86 allowable concentrations of environmental quality standards to 70 ng L⁻¹. However, in aquatic
87 systems, without any point anthropogenic or geological sources, total-Hg (THg)
88 concentration typically ranges from less than 0.05 to 12 ng L⁻¹ (Dennis et al., 2005; Driscoll et
89 al., 2007; Wiener et al., 2003). Because the maximum allowable concentrations of
90 environmental quality standards were much higher than naturally relevant concentration
91 levels of THg, national monitoring programs did not have the incentive to detect THg at
92 ambient concentrations. There is even less information about MeHg background levels,
93 which generally are between one and two orders of magnitude lower as compared to THg
94 levels (Driscoll et al., 2007). In this context, streams and rivers are of special concern
95 because very few studies have reported THg and MeHg concentrations at a wide
96 geographical scale (Balogh et al., 2002; Brigham et al., 2009; Eklöf et al., 2015; Lescord et
97 al., 2018; Marvin-DiPasquale et al., 2009; Tsui et al., 2009). While rivers and streams have,
98 however, been hypothesized to be important contributors to the global biogeochemical Hg
99 budget (Amos et al., 2014; Kocman et al., 2017; Schartup et al., 2015a; Sunderland and
100 Mason, 2007), quantitative information on Hg levels is largely lacking, especially in the
101 temperate zone. Filling this gap in knowledge is an important prerequisite in the ongoing
102 quest to reduce uncertainties associated with the global biogeochemical cycling of Hg and for
103 the development of remediation and risk management strategies within the context of the
104 Minamata Convention.

105 Freshwaters receive Hg from a variety of sources including atmospheric deposition
106 (Fitzgerald et al., 1998), release from industrial discharge (Bravo et al., 2015, 2014), and
107 lateral export from terrestrial sources (e.g. soils and wetlands) (Brigham et al., 2009; Eklöf et
108 al., 2012). Diffuse inputs from land management activities and remobilization of Hg
109 previously accumulated in terrestrial ecosystems can be an important source comparable in

110 magnitude to direct Hg anthropogenic inputs (e.g. industrial releases or mining activities) to
111 surface waters (Kocman et al., 2017). Long-term accumulation of Hg in terrestrial systems
112 has been explained by the strong binding between atmospherically deposited Hg and sulfur
113 sites within organic matter (OM) (Skylberg et al., 2006; Xia et al., 1999). In boreal
114 ecosystems, characterized by OM-rich soils, the strong correlation between THg and
115 dissolved organic carbon (DOC) found in several studies (Brigham et al., 2009; Driscoll et al.,
116 1995; Eklöf et al., 2012; Tsui et al., 2009) denotes that the mobilization of OM is an important
117 vector for Hg transport from catchment soils to surface waters (Eklöf et al., 2012; Grigal,
118 2002). Besides stream water DOC, the proportion of wetland area within the catchment and
119 the discharge have been used to predict THg concentrations in surface waters (Brigham et
120 al., 2009; Eklöf et al., 2015; Tsui et al., 2009). In contrast, the relationship between DOC and
121 MeHg remains poorly understood (Brigham et al., 2009; Tsui et al., 2009). The variation of
122 MeHg concentrations in aquatic ecosystems is indeed more complex, as MeHg is potentially
123 derived from numerous sources, not only terrestrial sources. In boreal streams, the wetland
124 proportion of the catchment has also been linked to MeHg freshwater concentrations
125 (Brigham et al., 2009). Wetlands, characterized by oxygen-depletion and low to intermediate
126 nutrient levels, are known to be important sites for MeHg production and a relevant source of
127 MeHg to inland waters (Schaefer et al., 2014; Tjerngren et al., 2012). Besides the
128 contribution of MeHg produced in wetlands to stream waters, in-stream MeHg formation
129 might also be an additional key source of MeHg (Balogh et al., 2002; Eklöf et al., 2015; Tsui
130 et al., 2009), especially in catchments lacking extensive wetland coverage. The methylation
131 of inorganic divalent Hg (IHg) to MeHg is primarily mediated by anaerobic micro-organisms
132 (Gilmour et al., 2013) and is controlled by temperature, availability of electron acceptors (e.g.
133 SO_4^{2-} and Fe^{3+}) (Bravo et al., 2015) and the molecular composition of OM, which influences
134 IHg-speciation (Graham et al., 2013; Jonsson et al., 2014), IHg-availability (Chiasson-Gould
135 et al., 2014; Mazrui et al., 2016; Schaefer and Morel, 2009; Schartup et al., 2015b) and
136 microbial activity (Bravo et al., 2017). Specifically, IHg methylation processes are enhanced
137 by the presence of algal OM (Bravo et al., 2017; Schartup et al., 2013) and fresh humic OM

138 leached from organic rich soils (Herrero Ortega et al., 2018). Indeed, elevated MeHg
139 concentrations have been linked to periods of high algal production (Gascon Diez et al.,
140 2016; Soerensen et al., 2017) and leaf fall (Balogh et al., 2002). However most studies
141 investigating the role of OM as a transport vector for Hg and/or a key factor controlling MeHg
142 formation have been carried out in the boreal biome. Consequently, there is still a high
143 amount of uncertainty in identifying factors governing THg and MeHg levels in stream waters,
144 particularly at a broader geographical scale.

145 The molecular composition of dissolved organic matter (DOM) is variable and can be
146 an important factor in predicting THg and potentially, MeHg (Bravo et al., 2017; Herrero
147 Ortega et al., 2018; Lescord et al., 2018; Noh et al., 2017; Schartup et al., 2015b, 2013). A
148 common fingerprinting approach used to characterize the bulk DOM composition in inland
149 waters is fluorescence spectroscopy (Fellman et al., 2010). The increasingly widespread use
150 of fluorescence can be attributed to its wide accessibility, ease of use, and low costs. Three
151 commonly used indexes (fluorescence index (McKnight et al., 2001), freshness index
152 (Parlanti et al., 2000) and humification index (Zsolnay et al., 1999)) are particularly useful for
153 distinguishing between DOM derived from different sources (McKnight et al., 2001), and
154 degrees of freshness and humification (Zsolnay et al., 1999). Likewise, commonly known re-
155 occurring peaks distributed across the fluorescence excitation-emission matrix (EEM) can
156 provide insights into sample specific DOM characteristics. Fluorescence has indeed
157 demonstrated the potential to reveal key DOM molecular characteristics associated with THg
158 and MeHg concentrations (Herrero Ortega et al., 2018; Lescord et al., 2018; Noh et al., 2017;
159 Schartup et al., 2015b).

160 The aim of this study was to identify the relationships between THg and MeHg
161 concentrations and DOM composition (sources and quality) across a diversity of European
162 streams. Unraveling the factors determining THg and MeHg background levels in the aquatic
163 environment at a broad geographical scale is crucial for a better understanding of Hg cycling.
164 For this purpose, we measured THg and MeHg concentrations, DOM concentration and

165 composition, anions and cations in filtered and unfiltered stream water at 29 sites with
166 contrasting catchment characteristic across a wide latitudinal gradient across Europe.

167

168 **2. MATERIAL AND METHODS**

169 **2.1 Organizational Framework**

170 Water sampling for THg and MeHg concentration measurements was organized
171 within the framework of the project called EuroRun: *Assessing CO₂ fluxes from European*
172 *running waters* (available from <https://freshproject-eurorun.jimdo.com/>). EuroRun represents
173 the first Collaborative European Freshwater Science Project for Young Researchers aiming
174 to create synergistic interactions among young and early career scientists across Europe.
175 Within the context of the collaborative EuroRun project, the EuroRun_Mercury initiative
176 included 12 teams distributed in 8 countries, which sampled 29 stream sites (Fig. 1). With
177 56% of participants being female, EuroRun_Mercury was gender balanced. The teams
178 represented different regions across Europe. For a specific region, each team selected
179 several sites with contrasting dominant land uses (i.e. forest or agriculture) and a wide range
180 of hydrological and watershed characteristics (Table S1).

181

182 **2.2 Sampling**

183 The sampling procedure was explained and demonstrated to one lead representative
184 from each team, who participated in a workshop performed in Sweden in September 2016.
185 All the material needed to collect water samples along with a detailed written protocol was
186 provided to participants during the workshop (see SI). The sampling took place during 3rd –
187 14th October 2016 and teams from Austria (AUT), Bulgaria (BGR), the Czech Republic
188 (CZE), Germany (DEU), Spain (ESP), France (FRA), Great Britain (GBR), Portugal (PRT)
189 and Sweden (SWE) collected water samples for this study (Fig. 1). The 29 sampled sites
190 were located across a wide latitudinal gradient (from 41°N to 64 °N) and altitude range (from
191 1 to 1560 m above sea level), and spanned a wide range of hydrological (discharge from 0.1
192 to 56 m³ s⁻¹) and catchment characteristics (Table S1). Each team provided hydrological

193 (discharge, stream wetted width and stream order) and catchment characteristics data (land
194 use in % and catchment area).

195 A total of 4 water samples were collected at each site. Amber borosilicate bottles
196 (VWR, 250 mL) were used to store water and analyze THg and MeHg species in filtered and
197 unfiltered fractions. A previous study determined the cleaning procedures suitable for THg
198 and MeHg analyses with these bottles (Cavalheiro et al., 2016). In the field, borosilicate
199 bottles were rinsed three times with filtered (0.45 μm , Sterivex-HV) or unfiltered water. For
200 dissolved (_D) THg_D and MeHg_D analyses about 250 mL of water were collected directly into
201 the amber borosilicate bottle, filtered (0.45 μm , Sterivex-HV) and acidified (1% v/v final
202 concentration; HCl Ultrex II, J. T. Baker) on site. A subsample of filtered water was placed
203 into 40 mL pre-muffled EPA borosilicate glass vials (VWR) with PTFE caps for analysis of
204 DOC concentration, DOM optical properties and concentration of anions and cations.
205 Unfiltered water samples were collected for determination of the total fraction (_T) of THg_T,
206 MeHg_T (acidified) and total organic carbon (TOC) concentration. Samples were kept at 4°C
207 and shipped to the laboratory by courier within 48 h. A subsample (10 mL) of unfiltered water
208 was filtered in the laboratory, and stored frozen for later determination of anions and cations.
209 Because four of the vials broke during transport, these samples could not be determined
210 (marked as not determined in Table 1 and S2).

211 Results for unfiltered water samples correspond to the total fraction (THg_T, MeHg_T
212 and TOC), whereas the results for filtered water samples represent the dissolved fraction
213 (THg_D, MeHg_D, DOC, DOM optical properties, anions and cations).

214

215 **2.3 Chemical Analyses**

216 **2.3.1 Analysis of Inorganic-Hg and Methylmercury**

217 Inorganic-Hg (IHg) and MeHg (monomethylmercury) concentrations (Table 1) were
218 measured using species-specific isotope dilution and capillary gas chromatography (Trace
219 GC Ultra, Thermo Fisher, Waltham, MA, USA equipped with a TriPlus RSH auto-sampler)
220 hyphenated to inductively coupled plasma mass spectrometer (Thermo X Series 2)

221 (Cavalheiro et al., 2016; Rodriguez Martín-Doimeadios et al., 2003). The detection limits of
222 this method are 0.02 ng L⁻¹ for IHg and 0.005 ng L⁻¹ for MeHg. The measurement error
223 (calculated by analyzing each sample three times) was less than 15% and 10% for IHg and
224 MeHg concentrations, respectively. Based on previous studies (Bravo et al., 2014;
225 Monperrus et al., 2004; Rodriguez Martín-Doimeadios et al., 2003), THg was calculated as
226 the sum of IHg and MeHg.

227 **2.3.2 TOC, DOC and DOM optical properties**

228 TOC and DOC concentrations were determined on unfiltered and filtered samples,
229 respectively. Prior to analysis, samples were acidified to a pH of 2 with 0.1 M HCl, and
230 analyzed using a Shimadzu TOC-V_{CPH} total carbon analyzer. Optical properties were
231 determined on filtered water using an Aqualog spectrofluorometer (Horiba), which measures
232 absorbance and fluorescence simultaneously. The fluorescence spectrophotometer has a
233 routine protocol before running samples that involves i) instrument validation (ensuring the
234 signal : noise ratio <20,000 counts ii) ensuring the excitation lamp is calibrated to a given
235 wavelength (maximum intensity at 467 nm), iii) the emission energy is calibrated using pure
236 water at an emission of 397 nm and an excitation of 350 nm. Fluorescence spectra were
237 collected across excitation wavelengths (Ex_λ) 250 to 445 nm at 5 nm increments and across
238 emission wavelengths (Em_λ) ranging from 300–600 nm. Bandpass and resolution were 5 nm.
239 All spectra were measured in a 1 cm quartz cuvette and automatically blank-subtracted,
240 corrected for instrument biases and inner filter effects using the FluorEssence™ software
241 (Horiba). Samples were measured for fluorescence at a 2 second integration time, with the
242 exception of five more optical dense samples from Great Britain and Sweden, which were
243 measured at 0.5 seconds. All intensities were normalized to 1 second integration time post-
244 analysis and the excitation-emission matrixes (EEM) were expressed in fluorescence units
245 (FU).

246 Peak picking was used to define the relative intensity of four common peaks across
247 the EEM including the Peak A (λ_{ex} : 260 nm, λ_{em} : 400–460 nm), Peak C (λ_{ex} : 320–360 nm, λ_{em} :
248 420–460 nm), and Peak M (λ_{ex} : 290–310 nm, λ_{em} : 370–410 nm) associated with soil

249 terrestrial DOM, as well as a protein-like peak T (λ_{ex} : 275 nm, λ_{em} : 340–350 nm) that is
250 considered to be of algal/bacterial origin (i.e. autochthonous to the aquatic environment)
251 (Fellman et al., 2010; Huguet et al., 2009). The summed area of total fluorescence (TF)
252 under the EEMs was used to normalize each of the four peaks, which were expressed as a
253 percent of TF (i.e. %Peak A = Peak A / TF x 100%). We also calculated three common
254 fluorescence indexes: i) the fluorescence index (FI, calculated as the ratio between emission
255 at wavelengths of 470 and 520 nm, at an excitation of 370 nm), an indicator of source being
256 more microbial (≈ 1.8) or terrestrial (≈ 1.2) (Cory et al., 2007; McKnight et al., 2001), ii) the
257 freshness index (calculated as the ratio of emission at 380 nm, divided by the emission
258 maxima between 420 and 435 nm, at an excitation of 310 nm) which reflects the age of OM,
259 with higher values representing more recently produced OM (Parlanti et al., 2000) and iii) the
260 humification index (HIX, ratio of peak areas across an emission spectra [435–480 nm / 300–
261 345 nm]), indicating the degree of humification (Zsolnay et al., 1999). The absorbance at 254
262 nm (A_{254}) was used to calculate carbon specific absorbance (SUVA_{254}), a commonly used
263 metric of the degree of aromaticity, as A_{254}/DOC concentration normalized to a 1 m path
264 length ($\text{L mg C}^{-1} \text{ m}^{-1}$) (Weishaar et al., 2003).

265 **2.3.3 Anions and Cations**

266 Inorganic analyses were conducted on a Metrohm IC system (883 Basic IC Plus and
267 919 Autosampler Plus). Anions (F^- , Cl^- , NO_3^- and SO_4^{2-}) were separated on a Metrosep A
268 Supp 5 analytical column (150 x 4.0 mm) fitted with a Metrosep A Supp 4/5 guard column at
269 0.7 mL min^{-1} using a carbonate eluent (3.2 mM Na_2CO_3 + 1.0 mM NaHCO_3). A Metrosep C4
270 column (250 x 2.0 mm) with a Metrosep C4 guard column was used for separation of the
271 cations (Na^+ , NH_4^+ , K^+ , Ca^{2+} , Mg^{2+}) with an eluent of 1.7 mM nitric acid and 0.7 mM dipicolinic
272 acid at a flow rate of 0.2 mL min^{-1} .

273 **2.4 Statistical Analyses**

274 Kolmogorov–Smirnov test and quantile–quantile plots were used to evaluate the
275 normality of the data. The distribution of THg concentrations were slightly skewed due to the
276 high concentrations measured at sites in Great Britain. The center and dispersion of THg

277 concentrations were summarized by the median and the inter-quartile range (IQR). DOM
278 optical parameters, MeHg and %MeHg met normality after being log-transformed. Parametric
279 tests were used to analyze linear correlations (Pearson) between DOM optical parameters,
280 THg and MeHg concentrations, and %MeHg and to compare Hg concentrations in the total
281 and dissolved fractions (t-test). All statistical tests were carried out in R 3.2.4 ([http://www.R-](http://www.R-project.org/)
282 [project.org/](http://www.R-project.org/)) (R Core Team, 2016).

283 Principle component analysis (PCA) was used as an initial diagnostic tool to examine
284 key relationships and identify strongly correlated variables including optical measurements
285 (i.e. %Peak A, %Peak C, %Peak M and %Peak T, SUVA₂₅₄, fluorescence index, freshness
286 index, humification index), TOC, sulfate, nitrate and calcium concentrations, land use and
287 stream order. This complete information was available for 21 of the 29 studied sites.
288 Accordingly, the samples with incomplete information (DEU1_1, DEU1_2, GBR2_2, FRA1_2,
289 PRT1_1, CZE1_1, GBR2_1 and ESP2_3) were excluded from the PCA analysis. All data
290 were log-transformed, scaled and centered prior to PCA analysis. PCA analysis were carried
291 out using the *prcomp* function of the package *stats* in R 3.2.4 (R Core Team, 2016).

292 Partial least squares (PLS) regression models were subsequently applied to predict
293 THg concentration (Y response variable) from DOM optical properties, water chemistry and
294 catchment characteristics (X predictor variables, Table S5 and Table S6). A second PLS,
295 excluding the sites with high THg concentrations (Great Britain, GBR1_1, GBR1_2), was also
296 tested (Supplementary information, Table S7). The PLS model performance was examined
297 based on the cumulative goodness of fit (R^2Y , explained variation), and the cumulative
298 goodness of prediction (or Q^2 , predicted variation). The variable influence on projections (VIP
299 scores) provided a means of assigning the importance of X variables on the overall PLS
300 model. X-predictor variables with an influence score ≥ 1 were considered highly influential,
301 between 0.8 and 1.0 moderately influential, and < 0.8 less influential predictors (Eriksson et
302 al., 1999). The PLS models were performed using the *pls* function of the *pls* package in R
303 (Mevik and Wehrens, 2007). Graphics were built in R 3.2.4 (R Core Team, 2016) and
304 modified to fit the journal requirements with Inkscape 0.92 (<https://inkscape.org/es/>).

305 THg and MeHg loads of both total and dissolved fractions (Table S3) were calculated
306 with the stream discharge value provided by the participants (Table S1) and the
307 concentrations measured in this study (Table 1).

308 **3. RESULTS**

309 **3.1 Mercury Levels (THg, IHg, MeHg and %MeHg)**

310 THg concentrations were consistently low among sites (Table 1). Median values for
311 total (THg_T) and dissolved fractions (THg_D) were 0.46 ng L⁻¹ (interquartile range, IQR: 0.28 –
312 0.8) and 0.24 ng L⁻¹ (IQR: 0.28 – 0.49), respectively. Streams in Great Britain (GBR) and
313 Austria (AUT) had the highest THg_T and THg_D concentrations (Fig. 2a, Table 1). THg_T
314 concentrations were generally higher than THg_D ($p = 0.004$), although both were significantly
315 correlated ($R^2=0.62$, $p = 0.04$; Table S3). IHg_T was also slightly higher than IHg_D (Fig. 2b).
316 MeHg_T concentrations ranged between 7.8 and 159 pg L⁻¹ and were similar ($p = 0.07$) to
317 MeHg_D (6.6 – 134 pg L⁻¹; Fig. 2c). The highest MeHg concentrations, for total and dissolved
318 fractions, were found at sites from Sweden (SWE2_1), Great Britain (GBR1_1) and Spain
319 (ESP1_3). The %MeHg was very similar in both the total (%MeHg_T) and dissolved (%MeHg_D)
320 fractions (Fig. 2d) but varied among sites, ranging from 1 to 43%. The highest %MeHg_T was
321 found at a Spanish site (ESP1_3). The median of THg_T and MeHg_T loads were 3.9 kg year⁻¹
322 (IQR: 0.6 – 6.8) and 0.3 kg year⁻¹ (IQR: 0.1 – 0.5), respectively.

323 Significant correlations were found between the total and dissolved fraction of THg,
324 IHg and MeHg (Table S3). As total and dissolved fractions were correlated, we used the total
325 fraction (THg_T, MeHg_T, %MeHg_T) for all the following statistical analyses (PCA and PLS).

326 **3.2 Water Chemistry**

327 DOC and TOC concentrations ranged between 0.9 and 18.5 mg L⁻¹ and 0.9 and 22.2
328 mg L⁻¹, respectively. DOC and TOC were significantly correlated ($R^2 = 0.99$, $p < 0.01$). The
329 highest concentrations were found in Great Britain (GBR1_1, GBR1_2) and Sweden
330 (SWE1_1) and the lowest in Spain (ESP1_2, ESP2_1) and Austria (AUT2_1, AUT2_2,
331 AUT2_3; Table S2). The concentrations of sulfate, a well-known electron acceptor for Hg
332 methylating microorganisms, varied largely and ranged from 1.3 (AUT1_1) to 731 (ESP2_3)

333 mg L⁻¹. About 50% of the studied sites presented sulfate concentrations lower than 12.6 mg
334 L⁻¹ (IQR: 6.8 – 66 mg L⁻¹). Spanish sites presented the highest sulfate concentrations (Table
335 S2). Nitrate concentration varied between 0.04 (GBR1_1) and 23 (ESP2_2) mg L⁻¹. The
336 median value for nitrate concentration was 1.9 mg L⁻¹ (IQR: 0.7 – 2.7).

337 **3.3 DOM Optical Characteristics**

338 Very distinct fluorescence signatures were observed between study sites, with four
339 illustrative EEMs representing contrasting signatures displayed in Figure 3. Sites from Great
340 Britain showed a strong optical signature representing terrestrially derived DOM, as indicated
341 by the high intensity of Peaks A and C, while in contrast, sites from Spain (ESP) and the
342 Czech Republic (CZE), with high Peak T intensities, represented DOM optical signatures of
343 waters typically enriched in autochthonous DOM (Fig. 3, Table S2). The wide range of bulk
344 DOM optical characteristics captured in this dataset can be attributed to the broad
345 geographical scale of this study.

346 Analyzed water samples also captured a wide range of DOM characteristics based on
347 each of the measured optical indexes. The SUVA₂₅₄, which is associated with the presence
348 of aromatic (Weishaar et al., 2003) and high molecular mass compounds (Chowdhury, 2013)
349 typically spans from <1 to a maximum of 6 L mg C⁻¹ m⁻¹ (Weishaar et al., 2003), and was
350 found to range from 2.4 (CZE1_2) to 5.2 (GBR1_1, GBR1_2) L mg C⁻¹ m⁻¹. HIX values
351 spanned from 2.2 to 25 (Table S2). The highest HIX values were measured in Austria
352 (AUT1_2) and Great Britain (GBR1_1, GBR1_2). Some of the lowest values were observed
353 in the Czech Republic (CZE1_2) and Austria (AUT2_2). While high HIX values (> 10) have
354 been linked to the presence of complex molecules and strongly humified organic material,
355 mainly of terrestrial origin, low values (<4) have been associated with autochthonous OM
356 (Huguet et al., 2009). The fluorescence index (FI) covered almost the full range of this index,
357 from a value of 1.3, representing terrestrial sources (degraded plant and soil OM; lower
358 values), in GBR1_2, to a value of 1.8, indicating microbial sources (including extracellular
359 release and leachate from bacteria and algae) in ESP2_2, ESP3_3 (Cory et al., 2007;
360 McKnight et al., 2001). The freshness index, typically associated with the contribution of

361 recently produced microbial-derived DOM (Parlanti et al., 2000), also spanned a wide range
362 from 0.34 to 0.91 and was positively correlated to the FI and negatively correlated to the HIX
363 and SUVA₂₅₄ (Fig. 4).

364 The wide distribution of DOM composition between sites indicated differences in the
365 source, processing pathways and biological reactivity of the DOM, as shown by the PCA
366 analysis (Fig. 5). The first principal component (PC1) explained 41% of the total variance
367 across 21 European sites (Fig. 5a). Positive loadings on PC1 were associated with high
368 TOC, A₂₅₄ and HIX, indicating a terrestrial DOM signature. In contrast, negative loadings of
369 PC1 were found for agricultural land use, NO₃⁻, SO₄²⁻, Ca²⁺, fluorescence Peak T and Peak
370 M, freshness index and FI, likely associated with fresh and recently produced algal and
371 microbial DOM (Ohno, 2002). Running waters from Great Britain (GBR1_1; GBR1_2),
372 Sweden (SWE1_1, SWE2_1) and Austria (AUT1_1, AUT1_2) were characterized by low
373 anions, cations and high concentrations of terrestrial humidified DOM (positive scores in PC1
374 Fig. 5b). In contrast, negative scores (Fig. 5b) of running waters from Spain (ESP) and the
375 Czech Republic indicated an enrichment in nutrients and fresh autochthonous OM. Also,
376 negative loadings for the percentage of urban and agricultural areas in the studied
377 catchments suggest that anthropogenic activities might enrich running waters in nutrients
378 and enhance primary production (Fig. 5a). The second component (PC2) explained 14% of
379 the variance. Positive loadings on PC2 were found for the fluorescence Peak A and the ratio
380 of the Peaks A to C (A/C). Both Peaks A and C have been linked to the presence of humic
381 substances (Fellman et al., 2010). However, an increase in the relative abundance of the
382 peak A compared to Peak C (A/C peak ratio) has been related to DOM processing, as Peak
383 C is lost preferentially to Peak A under both photo- and biodegradation processes
384 (Kothawala et al., 2012). Therefore, positive loadings of PC2 indicated terrestrial DOM that
385 had been processed (Fig. 5a). On the opposite end, negative loadings on PC2 were
386 associated with aromatic compounds (SUVA₂₅₄) (Weishaar et al., 2003) and terrestrial DOM
387 (Peak C) yet not strongly degraded (Kothawala et al., 2012).

388 **3.4 Predictors of THg, MeHg and %MeHg Concentrations**

389 A PLS model (PLS model I, Fig. 6) identified key factors driving THg_T concentrations
390 in running waters. From the 29 available sites, only 21 sites were included in the PLS model
391 which contained a complete set of required variables. To interpret the PLS loading plots, the
392 distance and positioning of Y variables (THg, MeHg or %MeHg) relative to X variables
393 indicated how well, or poorly, they related to each other. The greater the distance a variable
394 (X or Y) lies from the origin, the greater its overall influence. Variables situated close together
395 on the PLS plot are positively correlated, while variables opposite to each other are
396 negatively correlated. The proportion of variance in the X variables (i.e. DOM composition,
397 anions, cations and environmental factors) explained by the model is indicated by the R²X.
398 The R²Y value indicates the proportion of variance in THg explained by the model. With two
399 predictive components (R²X, 50%), the variance of THg concentrations was well explained
400 (R²Y, 83.2%) and relatively well predicted (Q², 60%) by the PLS model I (Fig. 6, Table S5,
401 Table S6). PLS model I indicates that while TOC and A₂₅₄ presented the highest positive
402 correlation with THg, freshness index, FI and agriculture were negatively correlated. When
403 not including sites highly influenced by terrestrial DOM inputs, GBR1_1 and GBR1_2, a
404 second PLS (PLS model II), with two predictive components (R²X, 40%), explained the
405 variance of THg_T concentrations relatively well (R²Y, 82%) but could not predict them (Q², -
406 2%) (Table S7).

407 It was not possible to predict a significant proportion of the variability in MeHg
408 concentrations using a PLS model with the measured variables. Nevertheless, the
409 concentration of MeHg was significantly correlated to %MeHg (Fig. 4). Additionally, the
410 %MeHg was significantly and positively correlated to Peak M (%), FI and sulfate
411 concentration and negatively related to HIX and THg_T (Fig. 4).

412

413 **4. Discussion**

414 **4.1 Hg levels in European running waters**

415 Our results provide new information on THg and MeHg concentrations in streams at a
416 European scale. THg concentrations across the studied European running waters were in the

417 same range as those measured in pristine streams of northern Sweden (Eklöf et al., 2012),
418 USA (Oregon, Wisconsin and Florida, Brigham et al., (2009); California, Tsui et al., (2009)
419 and Minnesota, Balogh et al., (2002)) and France (Cavalheiro et al., 2016) and were lower
420 than those reported for rivers typically contaminated by point sources (Baptista-Salazar et al.,
421 2017; Bravo et al., 2014). Therefore, none of the study sites appeared to be affected by point
422 sources of Hg.

423 Fluvial systems are an important transport pathway and ultimately deliver Hg
424 originating from catchment soils to the marine environment. At a broader scale, we attempted
425 to provide a rough estimate discharge of THg_T and MeHg_T of European rivers considering
426 median values obtained in this study and the total European river discharge (3100 km³)
427 (Shiklomanov and Rodda, 2004). Without considering point source of contamination, such as
428 chlor-alkali or mining activities, known to increase stream Hg concentrations, the estimated
429 European river discharge of THg_T and MeHg_T would be 1426 kg year⁻¹ (IQR: 868 – 2494)
430 and 97 kg year⁻¹ (IQR: 60 – 254), respectively. Particles are an important vector of Hg
431 transport in streams (Schuster et al., 2011) and can influence hydrologically mediated
432 processes such as sedimentation and/or remobilization (e.g heavy rain events) of Hg
433 previously deposited within river systems, which can largely affect THg_T and MeHg_T loads
434 (Baptista-Salazar et al., 2017; Moreno-Brush et al., 2016; Sunderland and Mason, 2007).
435 Measured THg and MeHg concentrations were notably higher in the total fraction compared
436 to the dissolved fraction, highlighting the role of particles in the transport and fate of Hg in the
437 studied fluvial systems. As precise hydrological data were unavailable here, and our
438 calculations are based on a single sampling campaign, and the discharge of THg and MeHg
439 only provides an initial rough estimate to be considered with care. In order to improve upon
440 current estimates of European Hg discharge into the oceans (Kocman et al., 2017), greater
441 monitoring of THg and MeHg is required across fluvial systems, particularly beyond the
442 boreal ecozone.

443 **4.2 Terrestrial DOM: An Important Source of THg in European Running Waters**

444 Using a multivariate approach comparing 21 streams spanning a wide geographical
445 area across Europe, we were able to discriminate between the relative influence of bulk
446 DOM optical characteristics and environmental variables on THg and MeHg concentrations.
447 PC1 of the PLS model showed positive correlations between the highly influential predictors
448 TOC and A_{254} with THg concentrations in the studied sites and suggests that terrestrial DOM
449 is an important driver of THg in streams (Fig. 6). Sites characterized by humic-rich soils, such
450 as those in Sweden and Great Britain (Jones et al., 2005), presented the highest THg_T and
451 THg_D concentrations. The positive correlation between terrestrial DOM and THg
452 concentration in boreal rivers and streams has been previously reported in the literature
453 (Brigham et al., 2009; Eklöf et al., 2012). As atmospheric Hg deposition during the industrial
454 era have led to the accumulation of Hg in organic rich soils (Johansson and Tyler, 2001), the
455 remobilization of Hg-enriched soil OM is an important process controlling THg concentrations
456 in streams (Baptista-Salazar et al., 2017; Brigham et al., 2009; Driscoll et al., 1995; Eklöf et
457 al., 2012; Kirk and Louis, 2009; Tsui et al., 2009) and lakes (Bravo et al., 2017).

458 It is noteworthy to highlight the lack of a significant correlation between TOC and
459 THg_T concentration ($p = 0.6$) when the sites with the highest TOC and THg_T concentrations
460 (SWE2_1, GBR1_1 and GBR1_2) were excluded from the statistical analyses. Also, a poor
461 predictability was obtained in a PLS model (PLS model II) built for THg_T excluding the sites
462 highly influenced by terrestrial DOM (GBR1_1 and GBR1_2, Table S7). Therefore, TOC, and
463 DOM components associated with terrestrial DOM, failed to predict THg_T concentrations in
464 southern parts of Europe (FRA, ESP, CZE, AUT, BGR and DEU). This suggests that other
465 Hg sources are relevant for the studied southern streams. Direct inputs of Hg deposited by
466 wet and dry atmospheric depositional processes (Brigham et al., 2009; Domagalski et al.,
467 2016; Lee et al., 2001) could contribute to THg_T concentrations in streams with low inputs of
468 terrestrial DOM. Besides atmospheric deposition, catchment characteristics and changes in
469 runoff may be relevant in determining THg_T in streams and rivers (Baptista-Salazar et al.,
470 2017; Domagalski et al., 2016). Therefore, our results confirm the role of terrestrial OM as an
471 important source of Hg for streams but highlight the uncertainties on the drivers of THg

472 concentrations at broader global scale when considering systems with low loads of terrestrial
473 OM.

474 **4.3 Autochthonous DOM and Nutrients: Important Drivers of MeHg Formation in** 475 **European Running Waters**

476 In this study, MeHg concentrations measured in running waters were in the same range as
477 those previously reported for Sweden (Skylberg et al., 2003), USA (California, Tsui et al.,
478 (2009); Florida, Brigham et al., (2009)) and France (Cavalheiro et al., 2016). Similarly, the
479 %MeHg measured in samples collected in Sweden and Great Britain were similar to those
480 previously reported in the literature (Brigham et al., 2009; Skylberg et al., 2003). In contrast
481 to THg, MeHg concentrations were not correlated to TOC, A_{254} or HIX (Fig. 4), suggesting
482 that terrestrial sources are not the main vector for MeHg in the studied streams. MeHg
483 concentrations have been related to the concentration of autochthonous OM in reservoirs,
484 lakes and in the Baltic sea (Gascon Diez et al., 2016; Noh et al., 2017; Soerensen et al.,
485 2017).

486 The %MeHg has been considered a proxy of net MeHg formation (Drott et al., 2008).
487 In the studied European streams studied here, the positive correlation between %MeHg to
488 Peak M and the FI indicated that autochthonous OM is important for MeHg formation. Peak
489 M, as well as Peak A and Peak C, have been generally described as humic-like peaks
490 (Fellman et al., 2010). Peaks A and C have been linked to DOM derived primarily from
491 vascular plant sources, aromatic and with a higher-molecular-weight fraction of the DOM pool
492 (Coble, 1996). In contrast, Peak M represents compounds less aromatic and of lower
493 molecular weight as compared to peaks A and C (Fellman et al., 2010). In a previous study
494 performed in freshwaters, strong correlations between Peak M and Peak T (protein-like) were
495 reported, but weaker relationships were observed between Peak M and Peaks A and C
496 (Kothawala et al., 2014). Indeed, Peak M was originally used as a marker for autochthonous
497 DOM from planktonic production (Fellman et al., 2010). A positive correlation of the %MeHg
498 with Peak M and the FI thus suggests that net MeHg production is linked to fresh
499 autochthonous OM. In contrast, the negative correlation between %MeHg and HIX suggests

500 that high concentrations of highly humified terrestrial DOM hampered Hg methylation
501 processes most likely by decreasing Hg bioavailability (Chiasson-Gould et al., 2014;
502 Soerensen et al., 2017) and/or bacterial activity (Bravo et al., 2017). High Hg methylation
503 rates have been associated with algal derived OM (Bravo et al., 2017; Gascon Diez et al.,
504 2016; Herrero Ortega et al., 2018; Mazrui et al., 2016). Our study provides further evidence
505 of the potential role of algal and/or microbial DOM in the *in situ* formation of MeHg in
506 streams.

507 The methylation of IHg to MeHg in aquatic systems has been primarily attributed to
508 the activity of sulfate-reducing bacteria, iron-reducing bacteria (FeRB), methanogens and
509 Firmicutes (Gilmour et al., 2013) in oxygen limiting conditions of sediments (Bravo et al.,
510 2017), hypolimnion (Eckley and Hintelmann, 2006) and settling particles (Gascon Diez et al.,
511 2016). As sulfate can be used as an electron acceptor by Hg methylating microorganisms
512 (Pak and Bartha, 1998), the positive significant correlation found between the %MeHg and
513 the sulfate concentration, suggests an important role of SRB in MeHg formation in running
514 waters and provides further evidence for the relevance of *in situ* MeHg formation.

515 Stream sulfate mainly originates from atmospheric deposition and/or geogenic
516 sources (Ledesma et al., 2018). Because sulfate release is accompanied by cation leaching
517 (Prechtel et al., 2001), high concentrations of both sulfate and cations (e.g. calcium, Table
518 S2) in Spanish sites indicate an effect of geological substrate of the river basin on the stream
519 water chemistry. Although it was not the focus of this work, these results underline the
520 relevance of geology and catchment characteristics on stream water properties and
521 ultimately also for Hg cycling.

522

523 **Conclusion**

524 Our findings indicated that streams enriched in terrestrial DOM, as typical of Northern
525 study sites, revealed the highest THg concentrations, suggesting the source was from
526 organic rich catchment soils. In contrast, Southern European streams were enriched in
527 nutrients and autochthonous DOM and although inputs of THg and terrestrial DOM from the

528 catchment were lower, we propose that the elevated MeHg concentrations in some of these
529 sites could be due to a higher *in situ* MeHg formation, likely related to enhanced microbial
530 activity, which is boosted by the availability of autochthonous algal/microbial derived DOM
531 and sulfate. Our results show the interplay between DOM components and THg and MeHg
532 concentrations in streams and provide new insights into Hg cycling in aquatic systems at a
533 broad geographical European scale.

534

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771

772 **Authors Contribution:**

773 AGB conceived the study. KA and PB coordinated the sampling and the teams involved in
774 the study. AGB and ET performed GC–ICP–MS analyses of Hg with guidance from DA. DNK
775 conducted fluorescence, spectrometry and DOC and TOC analyses. KA measured cations
776 and anions. AGB and DNK conducted all statistical analyses. AGB, DNK and KA built the
777 figures. AGB wrote the manuscript with significant assistance and comments from DNK, DA,

778 KA and PB. The rest of the co-authors carried out the sampling and contributed to the writing
779 of the MS.

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Table 1. Concentrations of inorganic-Hg (IHg), methylmercury (MeHg), total-Hg (THg = IHg+ MeHg) and the percentage of MeHg as THg (%MeHg) in the dissolved (filtered 0.45 μm : _D) and total (unfiltered: _T) fraction of the studied streams.

Country	Stream ID	IHg _D	MeHg _D	THg _D	MeHg _D	IHg _T	MeHg _T	THg _T	MeHg _T
		ng L ⁻¹	pg L ⁻¹	ng L ⁻¹	%	ng L ⁻¹	pg L ⁻¹	ng L ⁻¹	%
Austria	AUT1_1	1.04	12	1.05	1.2	0.96	16	0.97	1.6
	AUT1_2	0.8	19	0.82	2.3	0.93	19	0.95	2.0
	AUT2_1	0.14	38	0.18	21.1	0.52	54	0.57	9.5
	AUT2_2	0.06	7	0.06	10.4	0.05	8	0.06	13.0
	AUT2_3	0.2	9	0.21	4.4	0.34	26	0.37	7.0
Bulgaria	BGR1_1	0.19	24	0.21	11.4	0.26	27	0.28	9.8
	BGR1_2	0.2	39	0.24	16.4	0.23	31	0.26	12.0
Czech Rep	CZE1_1	0.45	53	0.51	10.4	nd	nd	nd	nd
	CZE1_2	0.24	7	0.25	2.8	0.64	112	0.75	15.0
Germany	DEU1_1	0.17	67	0.23	28.7	0.3	12	0.31	3.9
	DEU1_2	0.14	36	0.17	20.8	0.34	60	0.4	15.0
	DEU2_1	0.14	10	0.15	6.7	0.4	56	0.46	12.2
	DEU2_2	0.11	26	0.13	20.1	0.55	52	0.6	8.7
Spain	ESP1_1	0.3	67	0.37	18.2	0.37	89	0.46	19.3
	ESP1_2	0.22	16	0.24	6.7	0.13	14	0.14	9.7
	ESP1_3	0.16	122	0.28	43.0	0.21	159	0.37	43.1
	ESP2_1	0.14	38	0.17	21.8	0.1	31	0.13	23.5
	ESP2_2	0.53	52	0.58	9.1	0.86	76	0.94	8.1
	ESP2_3	0.5	53	0.56	9.6	0.14	65	0.2	32.5
France	FRA1_1	0.47	15	0.48	3.1	0.49	15	0.51	2.9
	FRA1_2	0.39	17	0.41	4.2	0.86	20	0.88	2.2
Great Britain	GBR1_1	2.17	134	2.3	5.8	2.66	124	2.78	4.5
	GBR1_2	nd	nd	nd	nd	2.31	24	2.33	1.0
	GBR2_1	0.19	45	0.24	19.2	0.33	88	0.42	20.9
	GBR2_2	0.14	17	0.16	11.1	0.27	15	0.28	5.4
Portugal	PRT1_1	0.25	20	0.27	7.5	nd	nd	nd	nd
Sweden	SWE1_1	nd	nd	nd	nd	0.44	89	0.53	16.8
	SWE2_1	0.48	135	0.61	21.9	0.7	153	0.86	17.8
	SWE2_2	0.13	26	0.16	16.2	0.15	30	0.18	16.4

Figure 1. Distribution of the 29 sampling sites across nine European countries (see exact coordinates in Table 1).

Figure 2. Box and whisker plots of the distribution of a) total Hg (THg) in the unfiltered (total) fraction THg_T and the filtered (dissolved) fraction THg_D (n = 27), b) inorganic Hg (IHg) in the total IHg_T and dissolved fractions IHg_D (n = 27), c) monomethyl-Hg (MeHg) concentrations (n = 27) in the total (MeHg_T) and dissolved (MeHg_D) fractions and d) the %MeHg (n = 27). Boxes represent 25th (Q1) and 75th (Q3) percentile. The median is indicated within the box. The lower whiskers represent the lowest value within 1.5 IQR of the Q1, and the highest within 1.5 IQR of Q3.

Figure 3. Excitation-emission matrixes from sites presenting contrasted DOM characteristics a) and b) show samples with an eminently terrestrially derived DOM rich in humic peaks A and C. Plots c) and d) display samples with a marked microbial or algal signature as indicated by the high intensities in the peak T region. Intensities are shown in fluorescence units.

Figure 4. Significant ($p < 0.05$, $n=21$) correlations between THg_T, MeHg_T, the %MeHg_T, DOM optical properties and stream characteristics. The ellipses have their eccentricity parametrically scaled to the Pearson correlation value (narrower ellipses represent higher correlation values). The orientation of the ellipses indicate negative (red) or positive (blue) correlations (scale on the right).

Figure 5. Principal Component Analysis (PCA) of European streams (n = 21) showing the explained variability arising from principle components 1 (PC1) and 2 (PC2) based on bulk optical characteristics of dissolved organic matter (DOM) and environmental variables including stream order and catchment cover, as presented in (a) the loadings plot, and (b) the corresponding scores plot displaying the 21 study sites.

Figure 6. Partial least squares loadings plot predicting the variability of total mercury (THg) concentration (Y variable, red) with several DOM optical properties and geographical and water chemistry characteristics as predictors (X variables). Fluorescence peaks are expressed as percentage of total fluorescence. Variables in blue were identified to be highly influential predictors of THg (with a variable influence on projection of ≥ 1.0)

Graphical Abstract. Conceptual framework of (a) streams characterized by high inputs of terrestrial DOM and high concentrations of THg and (b) streams enriched in microbial/algal DOM depicting high MeHg formation (%MeHg).