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Abstract

The study of the elemental and carbon isotopic composition of dissolved organic matter (DOM) is of great interest in deciphering the origin and transformations of organic matter in estuarine and coastal environments. Styrene-divinylbenzene copolymer (PPL) sorbent is currently the most commonly used material for the isolation of DOM from environmental samples. It is notably used for the development of molecular formula-based indices used to study DOM reactivity. However, any extraction procedure (including with PPL) will fractionate the DOM. If this fractionation is not well constrained it can lead to biased interpretations of the biogeochemical processes affecting DOM. In this work we investigate the fractionation effects of the PPL sorbent on the size class distribution of DOM and the carbon isotopic composition of the PPL retentate. The use of size exclusion chromatography, that does not require a pre-concentration step, allows a precise study of the fractionation of DOM (including aromaticity) by the PPL resin. Extractions performed on two types of humic substances dissolved in artificial seawater, using the PPL resin, showed high extraction yield (> 85%) and the stable isotopic carbon composition (δ^{13}C) of these compounds was successfully recovered. These results indicate that salinity is not a parameter affecting extraction yield on PPL sorbent. For a hydrophilic compound (atropine) the extraction efficiency was low (33%) and δ^{13}C signature was underestimated. Size exclusion chromatography measurements, in samples collected along a salinity gradient, demonstrate that the PPL sorbent strongly fractionates DOM. Although the DOM size class distributions in freshwaters and marine waters were initially different, their retentates were marked by similar size class distributions. This work demonstrates that PPL resin captures DOM compounds with less nitrogenous content and it seems to have a lower affinity for aromatic compounds of marine origin than of terrigenous origin. The study of DOM distribution in a macro-tidal estuary demonstrated the limitations

of PPL resin extraction in capturing an internal N-rich DOM production event at the time of sampling. Futhermore, the isotopic composition of the PPL resin retentate appears to depend on the extraction efficiency of the more hydrophobic compounds which changed along the salinity gradient. This study recommends careful interpretations of data that only rely on PPL extractions, particularly for works tracking the origin of DOM in estuaries and comparing DOM composition across ocean biogeochemical domains.

1. Introduction

Marine dissolved organic matter (DOM) is a large reservoir of reduced carbon (662 Pg, Hansell et al., 2009) involved in key aquatic processes. The main constituent of DOM is carbon (~50%) that supports the metabolism of heterotrophic bacterioplankton production (Azam and Hodson, 1977 ; Ducklow, 1999). DOM also consists of various heteroatoms making DOM an alternative bioavailable reservoir of nitrogen and phosphorus for marine micro-organisms (Lønborg et al., 2009 ; Stepanauskas et al., 2002). Thereby, DOM plays an integral role in the biogeochemistry of aquatic systems as well as in the biological carbon pump.

Historically DOM has been studied through the prism of dissolved organic carbon (DOC) since the early 1980. With major analytical advances (high-temperature combustion method), intercomparison efforts (Hedges and Lee, 1993) and international oceanographic programs (e.g. JGOF, CLIVAR) the global distribution of oceanic DOC was assessed in the mid 2000 (Hansell and Carlson, 2002, Hansell et al., 2009). DOC mapping demonstrated that its concentration was not uniform along the water column but exhibited concentration gradients across biogeochemical domains and a distribution strongly influenced by overturning circulation and deep sea mineralization (Hansell et al., 2009). However, despite the growing community interest in DOM, its study was still limited by analytical issues. Low ambient concentrations (down to 30 µM-C), salts, and wide diversity in the molecular composition (Zark et al., 2017) have been a barrier for decades to a molecular characterization of marine DOM.

For DOM isolation, solid-phase extraction (SPE) using historical amberlite sorbents (e.g. DAX 8 and 4) was developed early to define hydrophobic and transphilic fractions of DOM. However, their implementation and evaporation steps were often time consuming and required large sample volumes. In the late 2000, Dittmar et al. (2008) published a rapid and

63   simple method to isolate DOM from any aquatic matrix, which was a new step for the scientific

64   community. Among the 6 sorbents studied by Dittmar et al. (2008) the styrene divinyl benzene

65   polymer (PPL) was the most promising with extraction efficiency of 43% for DOC for deep sea

66   water and up to 65% for DOC in freshwater. This study permitted the emergence of the first

67   analyses of oceanic DOM composition along the entire water column and recently in different

68   biogeochemical domains (Broek et al., 2020 ; Hertkorn et al., 2013 ; Martinez-perez et al.,

69   2017 ; Medeiros et al., 2015 ; Osterholz et aL., 2021 ; Seidel et al., 2015). PPL is also widely

70   used for the isolation of soil organic matter (Patel et al., 2021) and for the development of

71   molecular formula-based indices (notably the aromaticity index of DOM) used to study DOM

72   reactivity (Zherebker et al., 2022).

73   SPE-DOC can be processed for stable isotope (δ^{13}C), a useful tool to trace the sources of DOC.

74   δ^{13}C of SPE-DOC helps to trace the estuarine mixing (Zhou et al., 2021), to distinguish the main

75   source of DOC (C3 vs C4 photosynthetic pathway) in estuary (Marques et al., 2017) and to

76   trace terrigenous inputs in coastal systems (Takasu et al., 2023) and in the ocean (Zigah et al.,

77   2017). δ^{13}C of SPE has usually the same or lower δ^{13}C than bulk seawater (Broek et al., 2017;

78   Zigah et al., 2017), suggesting that PPL resin selectively isolates compound groups and may

79   have difficulty in capturing the DOM derived from the degradation of marine phytoplankton.

80   Extraction with PPL resin has also been applied to culture experiments with the aim of defining

81   the molecular composition of DOM of phytoplankton or bacterial origin (Landa et al., 2013 ;

82   Liu et al., 2020) or to study the lability of oceanic DOM (Shen and Benner 2018; 2020). These

83   works have opened up a new field of research related to DOM ecology that focuses on the

84   complex interactions between organic compounds and microbial cells (Dittmar et al., 2021).

85   Because PPL resin has become the most popular sorbent, understanding the

86   representativeness of the DOM isolated using PPL resin is crucial. The few studies that address

87   the selectivity of this sorbent have shown contrasting results. While DOM of terrigenous origin

88   does not appear to be significantly fractionated during extraction (Raeke et al., 2016), the

89   sample matrix composition has been shown to affect the extraction efficiency of a given

90   compound (Johnson et al., 2017). Furthermore, incomplete elution of the PPL resin retentate

91   can induce a bias in the determination of stable and radiocarbon isotopic composition (Lewis

92   et al., 2020). It has also been shown that for seawater samples, the optical signature of the

93   retentate from a PPL resin is substantially different from that of the corresponding bulk

94 samples (Wünch et al., 2018). Another key issue is the high selectivity (chemically based) of

95 the PPL sorbent. Li et al (2017) analyzed retentates after SPE extractions using PPL resin for

96 Suwannee River and North Sea water samples. Their results showed high compositional

97 similarity in these two samples of different origin raising questions about the use of this

98 sorbent to study DOM modifications. Considering the results of Li et al. 2017 and the growing

99 number of studies reporting high similarities between DOM isolated across different oceanic

100 basins and depths (Broek et al., 2020), it is important to investigate the

101 selectivity/fractionation of DOM by the PPL sorbent during the extraction process using PPL

102 sorbent.

103 The objectives of our study are to define the representativeness of DOM isolated using a PPL

104 resin and to determine whether this type of extraction is adequate to capture internal

105 processes in estuarine systems and to trace the origin of DOM inputs (allochthones vs

106 autochthones). We first tested the accuracy of carbon stable isotope measurements of three

107 different types of organic compounds after their isolation on a PPL resin and measured the

108 stable carbon isotope composition in the PPL resin retentates. These experiments were done

109 to determine if SPE using PPL can be used to trace the origin of DOM in estuarine and marine

110 waters. Secondly, we studied the DOC size class distributions and DOM composition using size

111 exclusion chromatography (SEC) of estuarine samples before and after their passage through

112 the PPL resin in order to define the representativeness of the PPL retentate. SEC has the

113 advantage of not requiring a pre-concentration step and allows access to the DOM

114 composition without fractionation effects. This analytical tool has been successfully used for

115 the analysis of freshwater (Marie et al., 2015), estuarine (Dulaquais et al., 2018) and marine

116 waters (Fourrier et al., 2022) and allows in this study to determine precisely the fractionation

117 of DOM by the PPL resin in terms of size class and composition (including aromaticity and

118 nitrogen content).

119

2. Material and Methods

2.1 Sampling

11 samples were collected along the salinity gradient of the Aulne estuary-Bay of Brest estuarine system (Britanny, France) on May 31 2021. For salinities below 20 of practical salinity unit (PSU), sampling was operated from the R/V *Hésione* (INSU-CNRS-UBO) and was carried out 0.5 m below the surface with the arm fully covered by a plastic glove (92 cm, Polysem®) using acid cleaned high density polyethylene (HDPE) bottles. For salinities above 20, sampling was operated onboard of the R/V *Albert Lucas* (INSU-CNRS-UBO) and was done at 1 m depth using a Niskin bottle. All samples collected were filtered onboard the R/V *Albert Lucas* within two hours after sampling using precombusted 0.7-µm GF/F filters (Whatman®). The filtrates were then collected in acid-cleaned HDPE bottles, double bagged and stored in the dark at 4°C. It is worth noting that the cruise occurred during a low discharge period (9.1 m^3 s^{-1}). This water flow was preceded by a month of low water regime. This choice of water regime was made in order to have a residence time of the waters in the estuary (~15 days) significantly longer than the timescale of sampling (10 hours) allowing a better visualization of the biogeochemical processes occurring in the estuary. Salinity (S) and pH (NBS scale) were measured *in-situ* using a multi-parameter probe (Hanna Instruments© 9829) calibrated on the day of sampling. The accuracy of the measurements is ± 0.1 g kg^{-1} and ± 0.01 pH units respectively. Salinity and pH of the samples were remeasured in the lab at 25 °C. The marine end-member sample was collected in the Iroise Sea on October 22, 2018 during the FeLINE cruise (Riso et al., 2021) and stored at -20°C from collection until analysis. For all cleaning procedures and the preparation of all aqueous solutions we used ultrapure water (resistivity > 18.2 MΩ.cm, MilliQ Element, Millipore®) and HCl (Suprapur®, Merck).

2.2 Experimental and solid phase extraction procedures

The chart of the experimental procedure used in this work is shown in figure 1 and the details of the different steps are provided in the following sections.

For estuarine samples, solid phase extraction was operated once per sample. After acidification at pH 2 (Suprapur®, Merck), 0.5 L (S < 20) or 1 L of estuarine samples (S > 20) were passed through a 100 mg PPL cartridge (Agilent, Santa Clara, CA, USA) following the procedure described in Dittmar et al. (2008). The cartridges were first washed with 1 column volume of

methanol. Methanol (HPLC grade, Merck©) was rinsed from the cartridge with 5 volumes of ultrapure water acidified at pH 2 (HCl, Suprapur®, Merck) before the extraction started. Extractions were performed at a flow rate of 4 mL/min using a peristaltic pump (Watson Marlow © 205S) with TYGON® tubing. Before air-drying, the cartridges were rinsed with 5 volumes of ultrapure water acidified at pH 2 (HCl, Suprapur®, Merck) in order to remove salts. Then, after 5 min of air drying we started the elution of the cartridges. We used 1.5mL of HPLC grade methanol, which is within the range of the recommended volume (12 mL/g of resin) to avoid bias resulting from incomplete elution (Lewis et al., 2020). After elution, the methanol extracts were evaporated directly into smooth wall tin capsules (Elemental microanalysis ©) on a hot plate at 50°C. All of these steps were performed under a laminar flow bench (ISO-5). The tin capsules were then stored in precombusted glass vials in a desiccator until determination of elemental C and δ^{13}C (by Elemental Analyzer coupled to Isotope ratio mass spectrometer, EA-IRMS) within a week after extraction. It is worth noting that volatile organic compounds may be lost during the drying step inducing bias for elution recovery assessment.

For each sample, the DOC size class distribution was measured by size exclusion chromatography (SEC) before (bulk sample) and after (permeate, Non SPE-DOM) the sample passed through the PPL resin. The permeate is constituted of all the water samples that passed through the PPL resin during the extraction procedure. The retentate and eluate refer to the DOM retained by the PPL resin and recovered after a methanol elution. The extraction yield (EY%) for each class of compounds was determined according to equation 1. Since the retentate could not be injected in the SEC, the size class distribution of the retentate (SPE-DOC) was calculated by the difference between bulk and permeate DOC concentrations in each size class fraction. Elution recoveries (ER %) were estimated according to equation 2 by comparing the amount of C in the eluate (measured by an Elemental Analyze, EA , (Thermo Scientific EA Flash 2000) to the amount of DOC in the retentate (SPE-DOC multiplied by sample volume). We observed a contamination (with methanol) of the permeate during the extraction procedure for the sample at salinity 15.5. For this sample, the DOC recovery (50.7%) was estimated from the amount of C measured by an EA and considering 95% of ER. Recovery of the different fractions can not be assessed for this latter sample.

Equation 1 $EY(\%) = \left\{1 - \frac{[DOC]_{permeate}}{[DOC]_{bulk}}\right\} * 100$

180 **Equation 2** $ER(\%) = \dfrac{OC_{EA}}{\{([DOC]_{permeate} - [DOC]_{bulk})\} * V_{sample}} * 100$

181 With **EY** the Extraction yield; **ER** the Elution recovery; **[DOC]**$_{eluate}$ and **[DOC]**$_{bulk}$ the dissolved

182 organic carbon concentration measured by size exclusion chromatography before and after

183 the sample passes through the PPL resin respectively. **OC**$_{EA}$ *is* the amount of organic carbon

184 measured by the elemental analyser in the tin capsule after elution and evaporation of the

185 PPL resin; **V**$_{sample}$ the volume of sample that passes through the PPL resin.

186 For the evaluation of stable carbon isotopic accuracy, solid phase extractions were performed

187 for three certified referenced materials: Suwannee River Fulvic Acid (INTERNATIONAL HUMIC

188 SUBSTANCES SOCIETY (IHSS), SRFA 1S101F), Leonardite Humic Acids (International humic

189 substances society , LHA 1S104H) and Atropine (Elemental microanalysis , OAS 279202). These

190 compounds were selected by considering their isotopic signature and elemental composition

191 (Table 1). These materials were first dissolved in artificial seawater (final concentrations 2 mg

192 / L) prepared as described in Fourrier et al., 2022 (see supplementatry informations, SI).

193 Artificial seawater was then acidified to pH 2 (HCl) and 250 mL of these solutions were then

194 passed through the PPL resin, eluted and evaporated as for the estuarine samples.

195
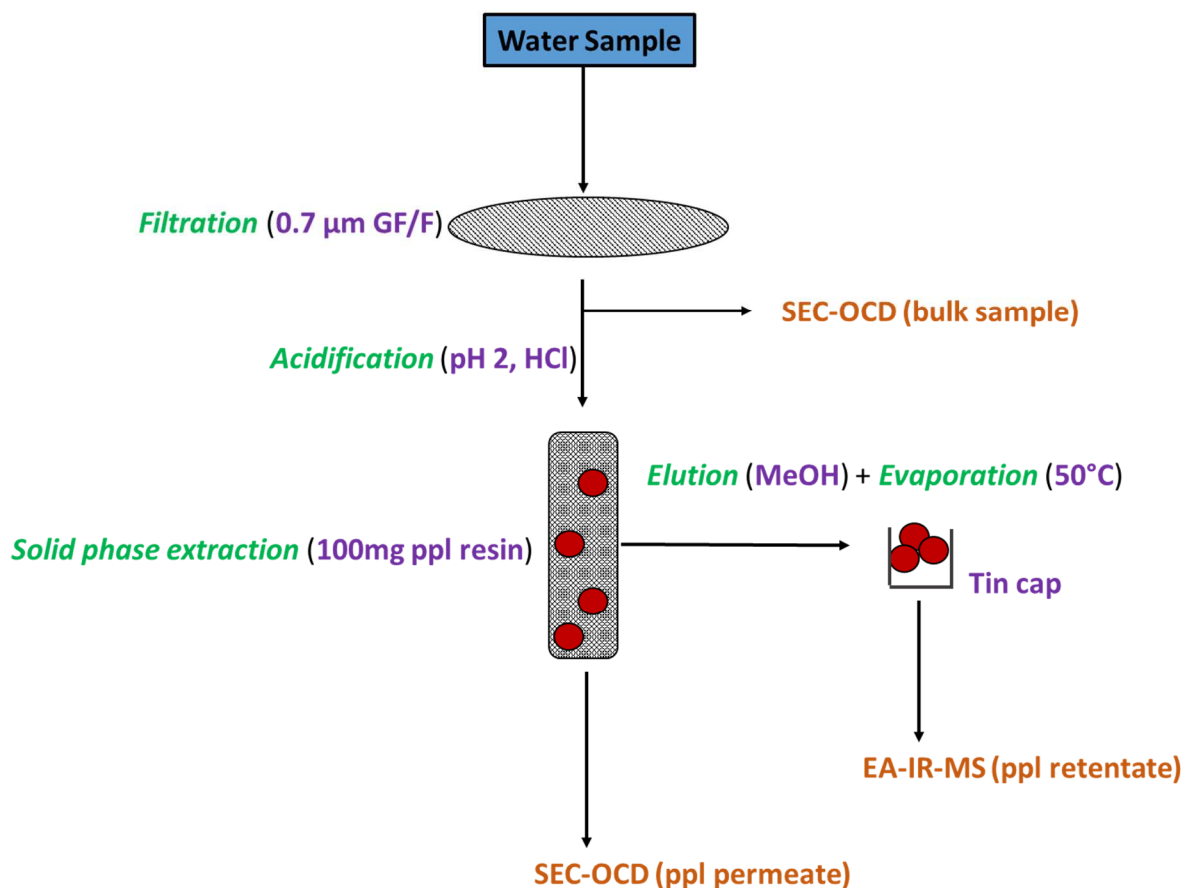
196 **Figure 1:** Chart of the experimental procedure followed in this study

197 2.3 DOM size class distribution

198 Measurements of the organic compound class distribution were performed by SEC coupled

199 with C, N and UV detectors (DOC-Labor®, Karlsruhe, Germany) as previously described by

200 Huber et al. (2011) for freshwater, and adapted for estuarine and marine waters by Dulaquais

201 et al. (2018b). All the chemicals used for SEC analyses were those described in Fourrier et al.

202 (2022). Repeated analysis of Deep Sea Reference samples (DSR, Hansell lab, Miami, USA

203 $[DOC]_{DSR}$ = 43.2 ± 1.7 µmol L^{-1}; n = 5; consensus value of lot #10-18: 43 – 45 µmol L^{-1}) ensures

204 an accurate determination of DOC. The determination of C:N ratios and of the percentage of

205 aromatic carbon (%C_{arom}) were conducted similarly as described in Riso et al. (2021) and

206 Fourrier et al. (2022) (see SI file for additional information regarding the procedures). The

207 device, equipped with two chromatographic columns (250 mm × 20 mm, TSK HW-50S, 3000

208 theoretical plates, Toso, Japan), permits the separation of DOM into six fractions of organic

209 compounds with an optimal resolution. These fractions were described in order of retention

210 as biopolymers (BP, high molecular weight compounds (HMW) > 10 kDa), humic substances

211 (HS, 0.5 – 10 kDa), building blocks (BB, 0.3 – 0.5 kDa), low molecular weight acids (LMW acids,

212 < 0.3kDa, charged), low molecular weight neutrals (LMW neutrals < 0.3 kDa) and the non-

213 chromatographable DOC (retained by the column at a pH of 7) called hydrophobic DOC (HOC).

214 The respective compositions of these operationally defined fractions are described in details

215 in Huber et al. (2011) and Dulaquais et al. (2018b). We provide additional information

216 considering the recent work of Fourrier et al. (2022). The BB fraction, initially thought as

217 degradation by-products of HS, may actually correspond to degradation by-products of BP;

218 LMW monoprotonic acids and LMW neutral compounds correspond to small-degradated

219 humic substances and small hydrophilic compounds, respectively.

220 2.4 Stable carbon isotopic measurements

221 Stable carbon isotope ratios and C content analyses were performed using an Elemental

222 Analyzer (Thermo Scientific EA Flash 2000) coupled to an Isotope Ratio Mass Spectrometer

223 (IRMS Thermo Scientific Delta Plus). Isotopic ratios were expressed in conventional δ notation

224 and were reported in parts per thousands (‰) as :

225 $\delta^{13}C = [((^{13}C/^{12}C)_{Sample} / (^{13}C/^{12}C)_{VPDB}) -1] *1000$

226   where $(^{13}C/^{12}C)$ $_{VPDB}$ = 0.0112372, the ratio of ^{13}C to ^{12}C in the international reference Vienna-

227   Pee Dee Belemnite (V-PDB) standard. Replicate analysis, using atropine as working standard

228   (calibrated to the VPDB) indicated an analytical precision of ± 0.1‰ and ±0.6% for $\delta^{13}C$ and

229   for C content, respectively.

230   The determination of bulk $\delta^{13}C$ composition (before solubilization) of the SRFA (- 27.6 ‰ ± 0.1

231   n = 3) and LHA (- 23.7 ‰ ± 0.1 n = 3) reference material were in keeping with the values (- 27.6

232   ‰ and - 23.8 ‰ respectively) provided by IHSS for these compounds (https://humic-

233   substances.org/elemental-compositions-and-stable-isotopic-ratios-of-ihss-samples/),

234   ensuring accurate of $\delta^{13}C$ measurements for natural organic matter.

2.5 Statistical analysis

236   T-tests were operated to determine if the PPL extraction procedure significantly affects the

237   initial $\delta^{13}C$ signature of the 3 organic compounds tested in this study (SRFA, LHA and atropine).

238   If *p-value* < 0.05 then the dataset are considered significantly different. The possibility of an

239   effect of the PPL extraction procedure onto DOM size fractionation was tested using a two

240   factor Wilcoxon test (two groups, permate and retentate) for each of the six size class

241   fractions.

3. Results and discussion

3.1 Accuracy of $\delta^{13}C$ measurement after extraction

244   Table 1 shows the $\delta^{13}C$ results (blank corrected) for the three referenced materials after their

245   solubilization in artificial seawater and extraction using the PPL sorbent. We found that the

246   PPL resin (≥ 88%) efficiently extracted SRFA and Leornadite Humic Acids. Their $\delta^{13}C$ isotopic

247   signatures were in agreement with those certified by the IHSS (table 1; *p-value* > 0.05). In

248   contrast, atropine was not extracted efficiently (33%) and we measured a $\delta^{13}C$ isotopic

249   signature (-19.9 ± 0.3; *p-value* < 0.05) significantly lower than the certified value (-18.1 ± 0.1).

250   On the one hand, our results demonstrate that humic substances of both freshwater and

251   terrigenous origin can be efficiently extracted from seawater matrix using a PPL sorbent. This

252   process does not induce isotopic fractionation of stable carbon isotopes. On the other hand,

253   our results suggest that hydrophilic molecules with low C/N ratio are not efficiently retained

254   by the PPL sorbent. This low extraction yield seems to induce an important bias for the $\delta^{13}C$

255   isotopic signature. Here we suggest that the $\delta^{13}C$measurement of a sample after solid phase

256   extraction using the PPL sorbent is strongly influenced by the type of compounds in solution

257   but not sensitive to the salinity of the sample.

258   **Table 1 :** *Certified elemental and stable carbon isotopic composition ($\delta^{13}C$) of the three certified referenced*
259   *materials used in this study. Recovery and $\delta^{13}C$ isotopic signature measured after their dissolution in artificial*
260   *seawater and extraction using a PPL sorbent.*

	SRFA	LHA	Atropine
Elemental O/C	0.60	0.37	0.18
Elemental H/C	0.99	0.7	1.37
Elemental C/N	85.0	60.5	17.0
$\delta^{13}C$ certified	-27.6	-23.8	-18.1
$\delta^{13}C$ measured in solid (n = 3)	-27.6 ± 0.1	-23.7± 0.1	-18.1 ± 0.1
Number of independent extraction	10	3	3
% recovery in artificial seawater	88	92	33
$\delta^{13}C$ measured after solubilization and PPL extraction	-27.8 ± 0.2	-23.9 ± 0.1	-19.9 ± 0.3

261   3.2 Retention yields for the samples collected in the salinity gradient

262   The extraction yield (EY) of the PPL resin was determined for DOC and for the different size

263   classes operationally defined by SEC analysis. The elution recoveries were all > 95% ensuring

264   no elution bias for EA-IRMS analysis of the retentate. Along the salinity gradient, DOC EY (Table

265   2) decreased from 52% in the Aulne freshwater (S = 0) to 35% in the marine sample (S = 35.15).

266   A similar decrease in EY from fresh to marine waters was initially observed by Dittmar et al.

267   (2008).

268   The different operational fractions exhibited varying EYs along the salinity gradient. On the

269   one hand, the EY% of the HS fraction decreased from ~60% to 45%. Those of the biopolymers

270   and LMW neutrals also decreased from freshwater to marine waters, from ~40% to ~13% and

271   ~30 % to ~1%, respectively. These results demonstrate the weak ability of the PPL sorbent to

272   capture these different hydrophilic compounds in marine samples. On the other hand, the EY

273   of the most hydrophobic organic compounds was enhanced for higher salinities (EY% range

274   90-100% for S>30) compared to the low salinity samples (EY% range 35-65%). Because riverine

275   and soil humic substances were accurately recovered in artificial seawater (S = 35, see section

276   3.1, table 1), salinity can not be considered as the controlling parameter for DOM adsorption

277   onto the PPL sorbent. Thereby, the changes of EY we observed along the salinity gradient

278   indicate changes in the DOM molecular composition. This selectivity of the PPL sorbent implies

279   that analyses of PPL retentate will only be representative of a fraction of DOM.

280   *Table 2 : Dissolved organic carbon concentrations (µM-C) measured in estuarine samples before and after*

281   *(permeate) the solid phase extraction for the 6 operationally defined fractions. Associated extraction yields (EY*

282   *%) for each fraction and total load of carbon in the PPL cartridge are provided. ND means not determined due to*

283   *a methanol contamination during the extraction procedure.*

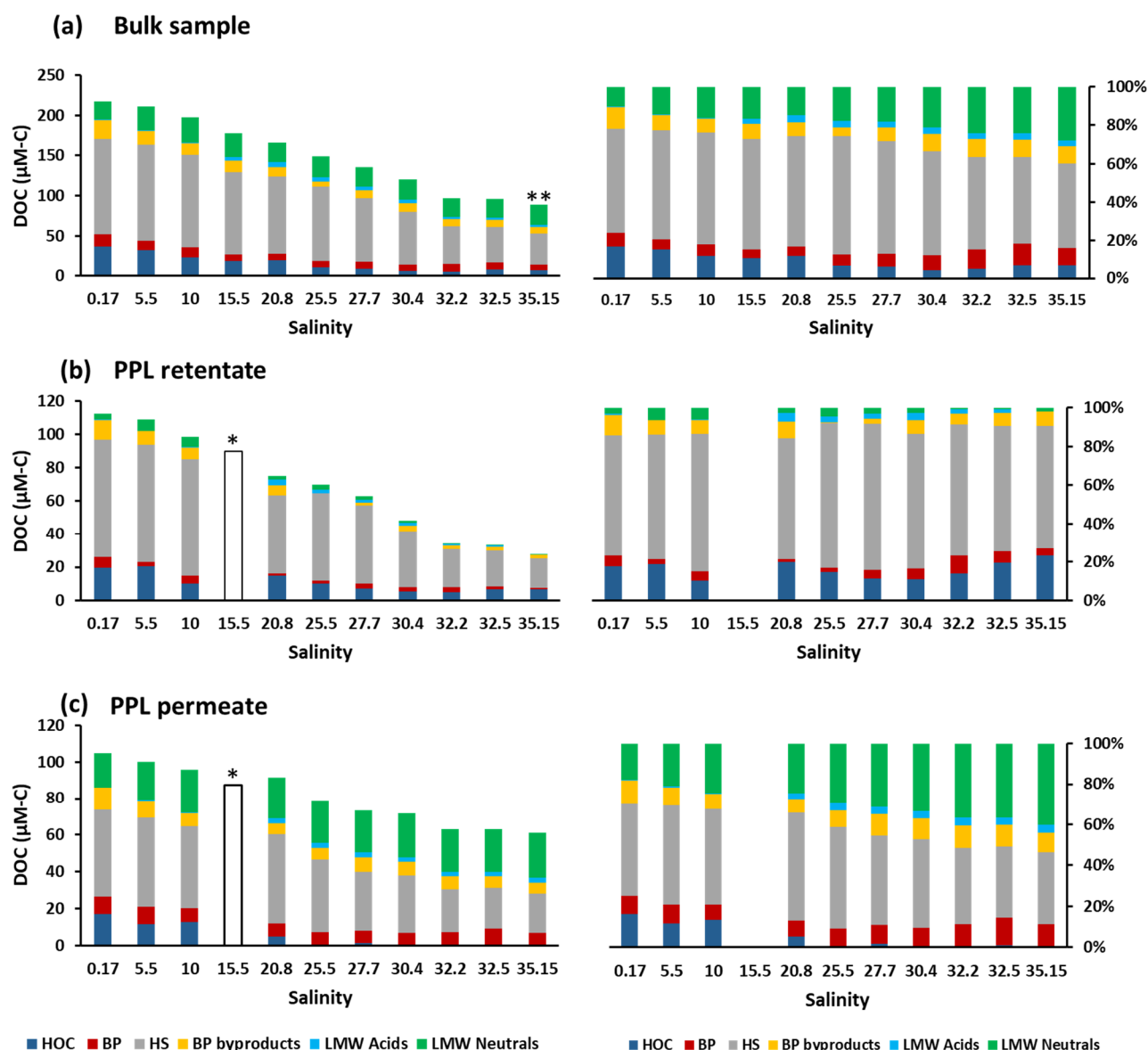| Salinity	Sampling location	Sample Volume (L)	Biopolymers (BP)			Humic substances (HS)			Biopolymers byproducts (BB)			Low molecular weight acids (LMW acids)			Low molecular weight neutrals (LMW neutrals)			Hydrophobic carbon (HOC)			DOC			Carbon in cartridge
			[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	[DOC] initial	[DOC] permeate	EY (%)	μmol C
0.17	48°12'57.8"N 4°04'30.0"W	440	15.7	9.3	40.8	117.6	47.2	59.9	23.9	12.2	49.0	1.0	0.3	70.0	22.5	18.9	26.0	36.9	17.1	53.7	230.0	105.0	54.3	55.0
5.5	48°13'16.3"N 4°06'59.1"W	494	11.8	9.2	22.0	119.3	48.7	59.2	17.2	9.1	47.1	0.8	0.6	25.0	30.0	21.1	29.9	32.2	11.6	64.0	211.3	100.3	52.5	54.8
10	48°14'43.3"N 4°07'11.9"W	462	12.2	7.3	40.2	114.8	44.6	61.1	14.1	7.0	50.4	0.8	0.5	37.5	32.1	23.5	32.9	23.9	12.8	46.4	197.9	95.8	51.6	47.2
15.5	48°14'47.6"N 4°09'48.3"W	470	8.2	nd	nd	101.9	nd	nd	14.0	nd	nd	4.5	nd	nd	29.5	nd	nd	19.3	nd	nd	177.4	nd	nd	44.5*
20.8	48°15'15.8"N 4°13'43.8"W	475	8.0	7.1	11.3	95.6	48.3	49.5	12.3	6.0	51.2	5.9	2.6	55.9	24.8	22.6	16.7	19.9	4.9	75.4	166.5	91.5	45.0	35.6
25.5	48°16'06.8"N 4°15'43.7"W	950	8.8	7.3	17.0	91.9	39.1	57.5	6.8	6.5	4.4	4.9	2.8	42.9	26.3	23.2	0.4	10.2	0.0	100.0	149.0	79.0	47.0	66.5
27.7	48°16'41.5"N 4°16'56.0"W	950	9.6	6.7	30.2	79.6	32.1	59.7	9.6	7.9	17.7	4.3	2.6	39.5	24.7	22.9	7.6	8.3	1.2	85.5	136.0	73.2	46.2	59.6
30.4	48°17'09.5"N 4°15'30.2"W	950	9.6	6.9	28.1	64.9	31.1	52.1	10.7	7.3	31.8	4.4	2.6	40.9	25.3	24.0	5.2	5.2	0.0	100.0	120.1	71.9	40.1	45.8
32.2	48°18'30.5"N 4°24'32.5"W	950	10.4	7.2	30.8	46.5	23.4	49.7	9.0	7.1	21.1	3.1	2.4	22.6	23.4	23.2	1.2	4.7	0.0	100.0	97.2	63.2	35.0	32.3
32.5	48°20'57.5"N 4°32'39.1"W	950	10.6	8.6	18.8	43.5	21.8	49.8	8.8	6.7	23.9	2.9	2.3	20.7	23.4	23.1	1.4	7.0	0.6	91.4	96.3	63.1	34.5	31.6
35.15	48°13'15.4"N 4°44'04.0"W	250	8.0	7.0	12.9	39.1	21.3	45.5	8.0	5.9	26.3	2.5	2.5	0.0	24.9	24.4	0.0	6.5	0.0	100.0	89.8	61.9	34.7	7.0

284   * Calculated using EA-IRMS carbon measurement and an elution recovery of 95% (ER = 95%). See text for details

285

286   3.3 DOC distribution and partitioning along the salinity gradient

287   The distributions of DOC and the six operationally defined size fractions along salinity gradient

288   are shown in figure 2a. DOC ranged from 234 µM-C in the freshwater sample (Aulne River) to

289   96.3 µM-C at S=32.5 (Bay of Brest). The North Atlantic surface water used in this work is a

290   sample collected from the Iroise Sea in 2018 (Riso et al., 2021) with a DOC concentration of

291   89.8 µM-C. At the time of sampling, DOC showed a nearly conservative distribution along the

292   salinity gradient (DOC = 235µM-C -3.9*Salinity ; n = 11; R^2 = 0.96) with a slight positive anomaly

293   at S > 20 (Figure S1). DOM partitioning was similar to those previously reported in the same

294   estuary (Dulaquais et al., 2018) with (1) the predominance of HS across the system (from 60%

295   of DOC at S < 10 to 45% at S > 32); (2) a decrease in HOC contribution of HOC from 15 % in

296   freshwater to 4 % in the most marine waters (36 µM at S = 0.17 to 4 µM at S> 32.5); (3) an

297   increase in the contribution of LMW neutrals with salinity (from 10% in freshwater to 25% in

298   marine waters), and (4) a low and near constant concentration of biopolymers (HWM DOC,

299   10.5 ± 2.3 µM, n = 11). With the exception of HOC, the mixing diagrams show non-conservative

300   behavior for all fractions studied (Figure S1), indicating that internal process were affecting

301   the distribution of DOM during the sampling period behind the quasi-conservative distribution

302   of DOC along the salinity gradient.

303

(a) Bulk sample

(b) PPL retentate

(c) PPL permeate

HOC BP HS BP byproducts LMW Acids LMW Neutrals

304

Figure 2: Dissolved organic carbon (DOC in µM-C) concentrations (left panels) and relative contribution
(right panels) in the six fractions operationally defined by size exclusion chromatography for (a) filtered
sample, (b) the PPL-retentate (SPE-DOM), (c) the PPL-permeate (Non SPE-DOM). It is worth noting that
a contamination with methanol prevents the analysis of the S=15.5 sample by SEC after the extraction
procedure (PPL permeate and retentate). ** Sample collected in 2018 and kept frozen until analysis.

3.4 Selectivity of the PPL sorbent

In order to study the representativeness of a PPL resin retentate we studied the size class
partitioning of DOC along the salinity between the PPL retentate (referred to as SPE-DOC
hereafter) and its permeate (referred to as non-SPE-DOC hereafter) .

The size class fractionations shown in Figure 2 clearly demonstrate a high selectivity of the PPL
sorbent for some fractions. The size class composition of the SPE-DOC samples was completely
different from that of the corresponding bulk samples (Figure 2) with an over-representation

317   of HOC and humic fractions in the SPE-DOC, at the expense of biopolymers and LMW neutrals

318   fractions. To further identify which size fractions are selectively retained by the PPL sorbent,

319   we determined the discrete (Figure S1) and average (with associated standard deviation)

320   contribution of each size fraction to the SPE-DOC pool and to the unextractable DOC (using

321   PPL) along the salinity gradient (Figure 3). Among the six fractions studied, five were retained

322   with a selectivity that induced a significant difference (Wilcoxon test) between the retentate

323   and the permeate (Figure 2, 3 and S1). Furthermore the high selectivity of the PPL sorbent

324   induced a similar distribution of SPE-DOC size classes between freshwater and the marine

325   waters, whereas before the extraction procedure, the samples had different compositions

326   (Figure 2 & 3).

327   The previous results demonstrate that during the extraction procedure, the size fractionation

328   of DOC changes. Thereby the DOC retained by the PPL resin is not representative of the bulk

329   sample. Such fractionation may lead to biased interpretations in the absence of additional

330   data (e.g. SEC measurements). Our results also showed that the selectivity of the resin was

331   however not total for a given fraction, suggesting that in a fraction operationally defined by

332   the SEC, the PPL resin is able to retain specific compounds according to their chemical

333   properties. To investigate this hypothesis, we compared the elemental C/N ratios and

334   aromaticity of compounds retained by the PPL sorbent to those that escape the extraction

335   procedure.
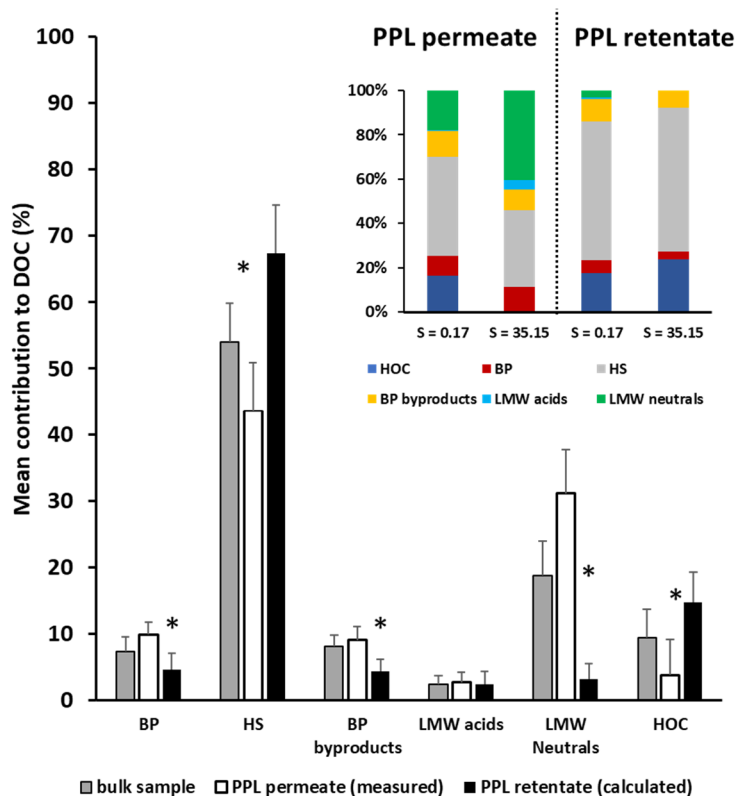
Figure 3: Mean DOC distribution in the bulk sample (grey bars), PPL permeate (non-SPE-DOC, white bars) and in the PPL-retentate (SPE-DOC, dark bars) for the 11 samples collected along the Aulne-Iroise Sea land-sea continuum. * indicate a significant difference between white and dark bars based on a two-factor Wilcoxon test (two groups, permate and retentate). Insert represents the DOM fractionation of PPL permeate and of PPL retentae for the freshwater (S = 0.17) and marine (S = 35.15) endmembers.

The C/N ratios measured in the PPL permeate were consistently lower than those measured in the samples before the extraction procedure indicating that the PPL resin has a low affinity for N-containing molecules. Since high nitrogen content is accepted as a proxy of DOM lability (Fourrier et al., 2022) and C/N ratio increases with DOM ageing (Walker et al., 2016); the ability of the PPL resin to capture labile and freshly produced DOM seems limited. This result is of particular importance because the decrease of C/N after PPL processing was observed in both humic substances (Figure 4a) and biopolymer (data not shown) fractions, which have different production pathways and labilities. Biopolymers (> 10kDa) can be considered as young and labile DOM derived from phytoplankton degradation (cell lysis). Humic substances are compounds that result from more intense processing (condensation, bacterial degradation), they can be considered as a more refractory fraction of DOM. Our results also show that the aromaticity of the biopolymers and humic substances fractions was also affected by the extraction procedure. We observed a much higher aromaticity of DOM in the PPL permeate

356 at salinities higher than 20 compared to the untreated samples (Figure 4b). This result

357 indicates that the PPL resin has a lower affinity for marine aromatic DOM than for

358 riverine/terrigenous aromatic compounds. Considering the low affinity of PPL for chemical

359 nitrogen groups (Figure 4a) our observation can be related to a higher proportion of aromatic

360 moities containing heteroatoms (e.g. O, N) in the marine DOM compared to the freshwater

361 DOM. Overall, such high selectivity for hydrophobic and aliphatic compounds at the expense

362 of aromatic and N-rich compounds strongly limits the use of the PPL resin to monitor the

363 production or to study the composition of labile DOM in marine waters.
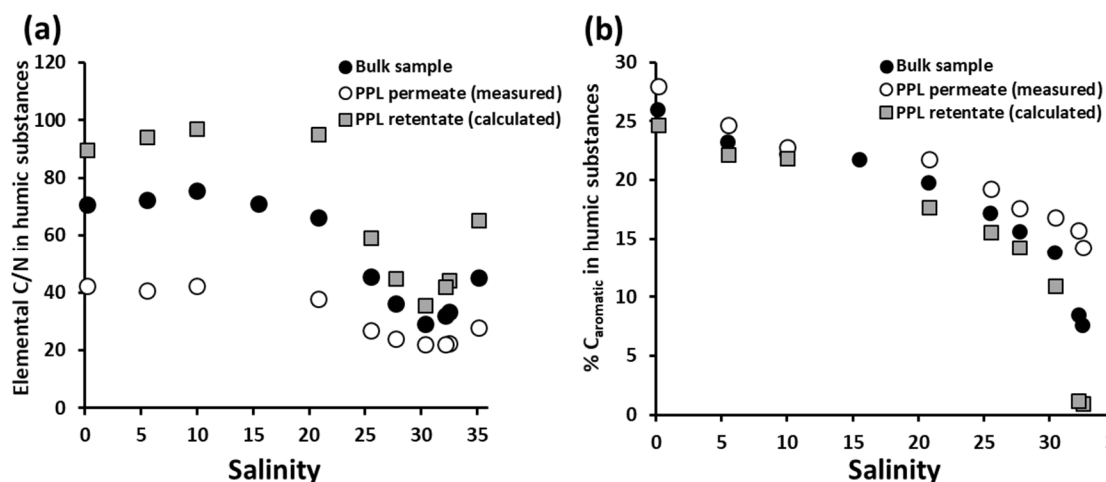
364

365 **Figure 4 :** Elemental C/N ratios **(a)** and percentage of aromatic carbon **(b)** measured in the bulk samples
366 (dark dots), in the permeate of a PPL resin (white dots) and calculated for the retentate of a PPL resin
367 (grey squares) for the humic substances fraction along the land-sea continuum of the Aulne-Iroise Sea.

368 ## 3.5 Isotopic composition of SPE-DOC along the estuary

369 Along the Aulne estuary, δ^{13}C values measured inPPL eluates ranged from -28.6 $\pm$ 0.1 ‰ in the

370 Aulne river freshwater to -24.2‰ $\pm$ 0.1 at S = 35.15. The depletion of δ^{13}C in the riverine end-

371 member is consistent with the terrigenous origin of DOM in the river (Opsahl et al., 1999).

372 Since δ^{13}C was not determined in the marine sample we must assume an isotopic signature of

373 -22.7 ‰ +- 0.1 in North Atlantic waters identical to the one reported in the work of Medeiros

374 et al. (2016) for North Atlantic SPE-DOC (using PPL). The increasing δ^{13}C isotopic signature we

375 observed along the estuary (Figure 5) is consistent with the increasing contribution of marine

376 waters. From these two end-members (e.g. Aulne River and North Atlantic), a mixing line can

377 be generated using a mass balance of the freshwater fraction (f) at each point according to

378 Equation 3.

379

Equation 3 $\delta^{13}C_{\text{SPE-DOCtheo}} = \{(\delta^{13}C_{Aulne} * [SPE\text{-}DOC]_{Aulne} * f) + (\delta^{13}C_{Atlantic} * [SPE\text{-}DOC]_{Atlantic} * (1\text{-}f))\} / \{([SPE\text{-}DOC]_{Aulne} * f)$ $+ ([SPE\text{-}DOC]_{Atlantic} * (1\text{-}f))\}$

With $\delta^{13}C$ SPE-DOC$_{theo}$ the theoretical stable isotopic carbon signature of the PPL retentate; [SPE-DOC]$_{Aulne}$ and [SPE-DOC]$_{Atlantic}$ the DOC concentrations of the PPL retentate in the Aulne river and in the Iroise Sea samples respectively; f the fraction of freshwater, f = 1 if S = 0.17 ; f = 0 if S = 35.15

Along the estuary, values of $\delta^{13}C$ (Figure 5, white dots) were close to the mixing line. Closer examination, however, indicates some deviations from this line, with heavier $\delta^{13}C$ values in the upstream part of the estuary (S < 21) and lighter $\delta^{13}C$ when S > 21. Given the uncertainties of both mixing line and samples, five data points drift significantly from the mixing line. Based on the SPE-DOC excess estimate (Equations 4 & 5), we used a mass balance (Equation 6) to determine the $\delta^{13}C$ signature of the apparent SPE-DOC excess ($\delta^{13}C$ SPE-DOC$_{excess}$, Figure 5, yellow dots). The calculated $\delta^{13}C$ SPE-DOC$_{excess}$ was -26.3 ± 0.2 for the two points in the salinity range 10-16 and was -27.8 ± 0.2 for the three points in the 25 - 31 salinity range.

Equation 4 $[SPE\text{-}DOC]_{excess} = [SPE\text{-}DOC]_{measured} - [SPE\text{-}DOC]_{theo}$

Equation 5 $[SPE\text{-}DOC]_{theo} = \{([SPE\text{-}DOC]_{Aulne} - [SPE\text{-}DOC]_{Atlantic}) / (S_{Aulne} - S_{Atlantic})\} * S_{measured}$

Equation 6 $\delta^{13}C$ $SPE\text{-}DOC_{excess} = \{(\delta^{13}C_{SPE\text{-}DOC\ measured} * [SPE\text{-}DOC]_{obs}) - (\delta^{13}C_{SPE\text{-}DOC\ theo} * [SPE\text{-}DOC]_{theo})\} / [SPE\text{-}DOC]_{excess}$

Interpretation based strictly on $\delta^{13}C$ data, may lead to a false conclusion and/or biased interpretations. The $\delta^{13}C$ SPE-DOC$_{excess}$ values in the salinity range 25-31 indicate a lighter organic $\delta^{13}C$ source that could be erroneously associated with terrigenous DOM inputs. Our study of the distribution of DOM size class by SEC clearly indicates an internal input of humic substances in this part of the estuary (Figure S1). At these salinities, the humic substances have aromaticities and C/N ratios much lower than the values measured for the Aulne end-member (Figure 4). Based on these properties, we relate this input of humic substances to an internal autochthonous production that may result from bacterial degradation of phytoplankton cells rather than to an external terrigenous input. If correct, these autochthonous humic substances should have a high $\delta^{13}C$ (phytoplankton origin) and increase the $\delta^{13}C$ signature of the DOM. Thereby there is a discrepancy between the SPE-DOC $\delta^{13}C$ values that suggest a terrigenous input and the SEC measurements that indicate autochthonous input.

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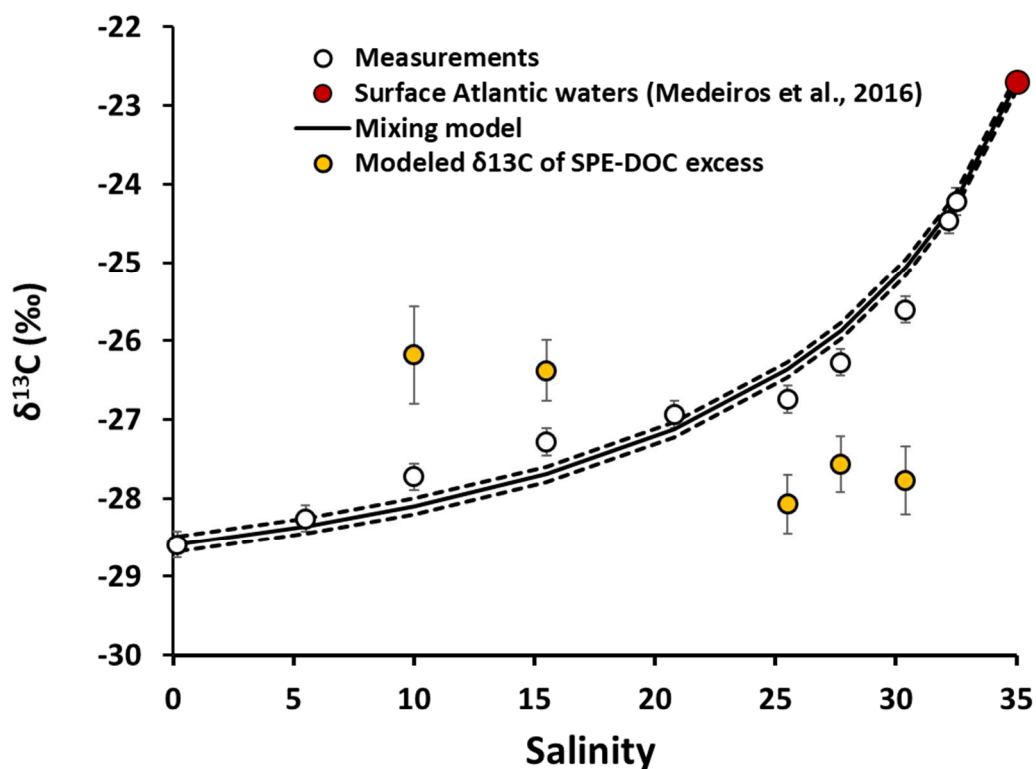
Figure 5: Stable isotopic organic carbon composition (δ^{13}C) measured in the PPL resin eluate (white dots) along the land-sea continuum of the the Aulne-Iroise land-sea continuum. Solid and dashed lines predict the conservative mixing and associated uncertainties calculated using the δ^{13}C value measured in the Aulne river (this study) and the δ^{13}C of the surface Atlantic ocean (red dot) determined by Medeiros et al. (2016). The δ^{13}C associated with excess SPE-DOC (yellow dots) were calculated by mass balance between δ^{13}C measurements and the excess SPE-DOC. See text for details of calculations.

Two distinct processes can explain this discrepancy. First, autochthonous DOM production in the salinity range 25-31 resulted in an input of N-rich humic substances. Considering their aquatic origin, these humic substances produced in the estuary are probably enriched in ^{13}C. These N-rich humic substances are weakly retained by the PPL resin (Figure 4a) and thus not available for the δ^{13}C measurement. Second, the extraction efficiency of HOC compounds increasing along the salinity gradient (Figure 6) may have affected the δ^{13}C values. Indeed, the PPL retained HOC very efficiently (> 95%) at salinities above 25, the extraction yield was much lower (35-65%) at salinities below 20. These HOC are primarily river-borne (Figure 6) are likely characterized by a light δ^{13}C consistent with their terrigenous origin. Therefore, any changes in the recovery of these compounds by the PPL sorbent will also affect the SPE-DOC δ^{13}C measurements.

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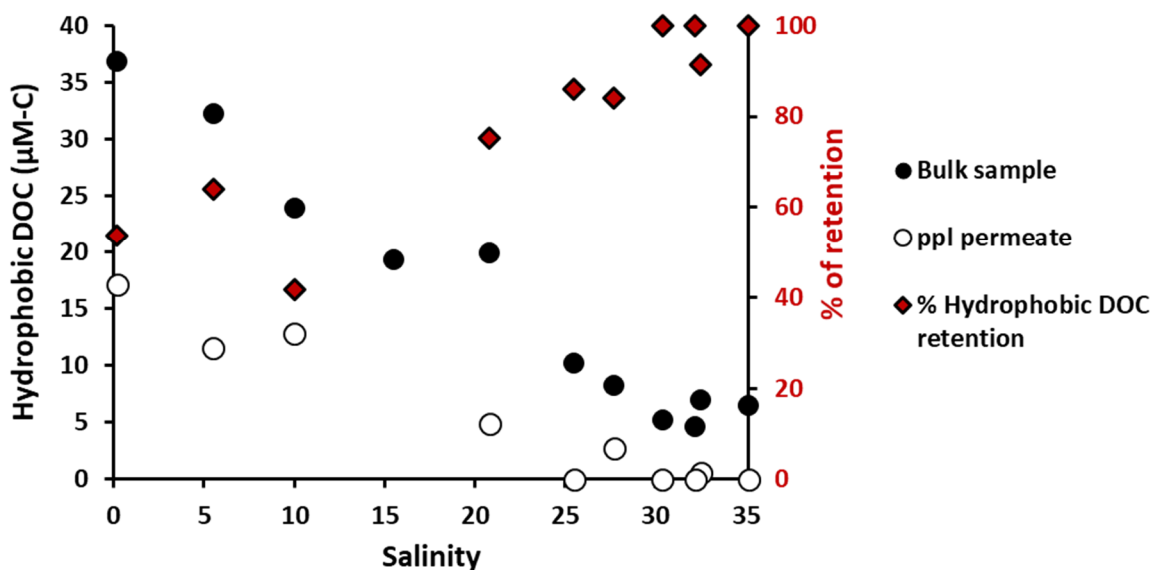
Figure 6: Hydrophobic DOC concentrations (left axis) in the bulk samples (dark dots) and in the PPL-permeates (white dots) measured along the Aulne-Iroise Sea land-sea continuum. The associated percentages of hydrophobic DOC extraction yields (red diamonds, right axis) increase from fresh to marine waters.

3.6 Implication for oceanic studies

Extraction with PPL as sorbent is commonly used to isolate DOM in order to trace molecular changes in DOM through biogeochemical processes (Osterholz et al., 2021). Here, we observed that extraction with a PPL sorbent alters the size class distribution of the initial DOM and that this resin is not optimal for capturing N-rich or aromatic marine DOM (Figure 4). In addition, we observed a high selectivity of the PPL resin for specific DOM fractions. This selectivity, already reported in other studies (Li et al., 2017; Wünch et al., 2018), generates a quasi-constant composition of the DOM isolated by the PPL resin whether in freshwater or marine waters (Figure 2 and 4). The PPL sorbent is often reffered as having a high affinity for refractory DOM compounds, which would make this sorbent effictive for studying this DOM fraction. It is clear that an PPL extraction of DOM with PPL in the deep ocean will trap a substantial part of the refractory DOC (up to 60%), however any analysis at the molecular level of the retentate from a PPL resin cannot be assigned to the intrinsect signature of refractory DOC. This is supported in particular by the work of Broek et al. 2020. These authors observed significant compositional differences between bulk DOM and PPL DOM retentates in term of C/N ratio, $\delta^{13}C$ and $\Delta^{14}C$ in the deep Pacific waters. At these Pacific depths, DOM there can be

448 considered fully refractory (Fourrier et al., 2022), so the existence of compositional differences

449 between retentate from a PPL resin and untreated sample demonstrates that the PPL resin

450 captures only a fraction of the refractory DOM. Furthermore in a controlled laboratory

451 experiment conducted by Wienhausen et al., 2017, the PPL resin retained a portion of the

452 freshly produced (labile) DOM, again supporting that this sorbent fractionates the DOM not

453 in terms of lability but in terms of chemical properties.

454 These important results provide evidence that PPL resin fractionates DOM through chemical

455 properties and that DOM refractability can not only be defined as the chemical properties of

456 compounds retained by a PPL resin.

457 Due to the high selectivity of the PPL resin, we suggest that both labile and refractory DOM,

458 can be isolated using a PPL resin. Consequently, a DOM isolated using the PPL resin will have

459 the same chemical properties and molecular composition (in broad terms) in any aquatic

460 system. As a result, the refractory nature of DOM can not be assigned solely in terms of

461 chemical composition. This hypothesis is supported by systematic fractionation of DOM that

462 we observed in PPL retentated from marine, estuarine and freshwater (Figure 2) while the

463 main origin of DOM is still discernable using carbon isotopes (Figure 5). This is also supported

464 by the accumulation of recent studies showing extraordinary homogeneity in DOM

465 composition from surface to the deep ocean , between oceanic basins and in different

466 environments (Broek et al., 2020 ; Li et al., 2017 ; Osterholz et al., 2021). Analysis of PPL

467 retentates by any powerful technique (e.g. Fourier Transform Ion Cyclotron Resonance Mass

468 Spectrometry *;* Nuclear Magnetic Resonance*)* provides useful information to study this

469 defined fraction. However, one should not lose sight of the fact that the PPL retentate is not

470 representative the refractory DOM alone but fractionate DOM in both in the refractory and

471 non-refractory pool.

4. Conclusions

473 We studied the fractionation of DOM by solid-phase extraction with a PPL sorbent along an

474 estuarine system. Our study confirms that the composition of the material is the main

475 parameter controlling the extraction efficiency of DOM by the PPL resin. PPL sorbent has a

476 high affinity for aliphatic hydrophobic compounds and retains poorly high molecular weight

477 compounds as well as those enriched in nitrogen. Salinity does not seem to affect the

478 extraction significantly. In this work we have demonstrated that at low extraction yields (<

479 33%) of a compound, the measurement of $\delta^{13}C$ in the retentate of a PPL resin is in error. For

480 yields > 88% the $\delta^{13}C$ measurement is valid.

481 The study of the size class fractionation of estuarine samples before and after their treatment

482 on a PPL resin allowed us to observe that (i) the composition of a PPL resin retentate was not

483 representative of the original sample, (ii) the distribution of DOC by size class was constant in

484 the different retentates without taking into account the origin of the sample (freshwater and

485 marine water). We observed a high selectivity of the PPL resin based on chemical properties

486 (C/N ratio, aromaticity). This selectivity was observed for both refractory (humiques

487 substances) and semi-labile (biopolymers) organic matter.

488 The carbon isotope study showed that the extraction efficiency of the compounds strongly

489 influenced the $\delta^{13}C$ data obtained from the retentate analysis of a PPL resin. In this work, a

490 high retention of the most hydrophobic compounds and a low extraction of humic substances

491 enriched in nitrogen and produced in the estuary induced an underestimation of the $\delta^{13}C$ at

492 the highest salinities (S > 20).

493 This work shows that without control of the extraction process, biased interpretations can be

494 made based on the analysis of a PPL resin rententate alone. We call for a cautious

495 interpretation of the DOM data from PPL extraction because this sorbent strongly fractionates

496 the DOM pool on chemical and molecular properties and not on the lability.

Acknowledgement

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