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Recommendations and good practices for dissolved organic carbon (DOC) analyses at low concentrations

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ABSTRACT

Numerous protocols for dissolved organic carbon (DOC) measurements on natural water are used in the literature. An ISO protocol for the determination of DOC exists since 2018, but it is certified for DOC values ≥ 1 mg L^{-1}, while many publications report DOC values much lower. In addition, this ISO protocol does not include indications on vials cleaning, filtering material, and type of caps and septa to be used. The purpose of this study was to evaluate protocols for measurements of low DOC concentrations (≤ 1 mg L^{-1}). The effect of the sample container, type of septum, filtration material, nature of acid used for storage, and matrix effects on DOC concentration were evaluated.

- The use of glass vials decontaminated at 450 °C or 500 °C for at least 1 h, 0.45 µm hydrophilic polytetrafluoroethylene (PTFE) membranes previously rinsed with 20 mL ultra-pure water and HCl acidification gives the lowest DOC contamination,
- Sulfides (ΣH_2S), sodium (Na^+) or calcium (Ca^{2+}) do not induce high matrix effect for the analysis (≤ 10%),
- At low DOC concentrations (≤ 1 mg L^{-1}), the use of pierced PTFE septa with acidified samples induce slight DOC contamination after storage at 4 °C, and dramatic contamination after storage at -18 °C.

Specifications table

Subject Area:	Environment
More specific subject area:	Rivers, Lakes, aqueous samples
Method name:	Sampling and storage recommendations for dissolved organic carbon (DOC) analyses at concentrations ≤ 1 mg L^{-1}
Name and reference of original method:	ISO 20,236:2018(F)
Resource availability:	N/A

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Method details

Introduction

Dissolved organic carbon (DOC) is a key parameter to understand carbon cycle in the environment. DOC concentrations in river basins, glacier melt waters [1], permafrost [2,3], or groundwaters [4] are partly controlled by major driving parameters such as seasonal effects and climate change. Annual floods and hydraulic flushes influence DOC variations in rivers [5], as well as human activities in estuaries [6–10] or in rainwaters [11]. DOC is also linked to trace metals mobility [12] and especially to the biogeochemical cycle of the toxic element mercury [13,14].

Because of these environmental implications, a reliable method to determine DOC in samples is fundamental, and requires a careful control at all the steps, from sampling to storage until the analyses. DOC is a parameter monitored for water quality and even if a protocol referred as ISO 20,236:2018(F) exists since 2018, analysis of the literature show that various practices are actually observed (see details hereafter, Tables 1 and 2). Cleaning procedure for sample containers, precautions to be taken with the vial closures (*i.e.* cap and septa), as well as the type of membrane are not included in the ISO protocol, apart from the recommendation to use a 0.45 μm cut-off to collect DOC. In addition, this ISO protocol only mentions two types of acids (H_2SO_4 and HCl) and deals with DOC concentrations higher than 1 mg L^{-1}, whereas numerous studies report much lower concentrations [1,3,5,10,11,15–23].

Vials and decontamination

Studies implying DOC analyses are numerous: nearly 15,500 publications can be listed since 2018, of which around 50% are in the field of environmental sciences (source: Web of Science). Some of them, including 20 recent ones, are listed in Table 1. Even if it is well known that decontaminated glass vials are classically used to store the samples before DOC analyses, no systematic protocol is followed regarding the choice of the vials (glass *versus* plastic) and their decontamination before use. In older articles, the use of either polytetrafluoroethylene (PTFE) vials [22], polyethylene (PE) vials [11], or glass vials [24] was reported. In the most recent publications (*i.e.* from 2018 to 2023), studies refer to the use of plastic vials, including high-density polyethylene (HDPE) [2], or polycarbonate [25], but also glass vials that are used commonly [3,9,10,15]. Plastic bottles have the advantage of being easy to transport for sampling in remote areas but on the other hand, glass vials can be fitted directly onto the autosampler of the DOC analyzer, which limits contamination during sample transfer. When using plastic vials, decontamination was made under acid washes [2,8,11,19,26], which can last up to one week [1]. When glass vials were used, DOC decontamination was mainly carried out by calcination but here again, methods are multiple, ranging from 4 h of calcination [9] to 12 h [19], at temperatures from 450 °C [9] to 550 °C [24]. Sometimes, the decontamination was not specified, suggesting that other protocols might have been used. As opposed to decontamination involving calcination or the use of nitric acid bath, Campos et al. [24] found that the best glass vial decontamination procedure was obtained using a bath containing Fe^{2+} and H_2O_2, with a lowest residual DOC concentration of 6.83 μmol L^{-1} (*i.e.* 0.082 mg L^{-1} of C according to Garcia-Martin et al., 2021).

Sample filtration and bacterial inhibiting agent

While glass fiber filters (GF/F) with a size cut-off of 0.7 μm are widely used in the literature, glass fiber filters (GF/D) at 3.9 μm are also occasionally reported [6,27] (Table 1). After sampling, the advantage of using such filters is that they can be analyzed to determine particulate organic carbon (POC). However, their drawbacks are their need for decontamination, *i.e.* time-consuming methods related to calcination, rinsing and drying.

Moreover, there are nominal filters, *i.e.* the value of 0.7 μm is the apex of a Gaussian curve of the distribution of the filter's cut-off points. These filters are different from the classical absolute membranes at 0.22 or 0.45 μm cut-off that are used for trace metal sampling [14,28,29]. In other studies, polymer membranes such as polyvinylidene fluoride (PVDF) [26], PTFE [30], nylon [15], or cellulose acetate (CA) [8] at either 0.22 or 0.45 μm were used. A specific problem relates to the filtration-based operational distinction of dissolved *versus* solid reactive substances. Thus, the use of a unique filter threshold value for all investigated elements (metals, DOC and other water chemical parameters) appears to be a prerequisite for comparing dissolved loads in modeling approaches [14].

To inhibit bacterial activity and its inherent DOC consumption, samples were often acidified mainly with phosphoric acid (H_3PO_4) [7,11,15,17,19,22], but also hydrochloric acid (HCl) [5,6,27] or more rarely sulfuric acid (H_2SO_4) [7]. The combination of $HgCl_2$, H_2SO_4 and NaN_3 or only one of them has also been reported [31,32]. The acid grade was seldom specified, except for one study, where ultra-clean quality H_3PO_4 was used [17].

Before analysis, samples were kept either at 4 °C [3,32] or frozen [15,23,26,33]. But here again, either for acidification or for storage, a majority of studies does not specify these technical details, suggesting that different procedures might have been followed.

Preservation techniques

Few published studies are devoted to a precise of the preservation techniques, and most of them are relatively recent, *i.e.* from 2015 (Table 2). The one from Tupas et al., [19] was cited by Walker et al., [34] as the first rigorous research on this topic. Tupas et al., showed no differences in DOC values in seawater samples stored up to 5 months, either unacidified, frozen in HDPE or acidified at 4 °C in sealed glass ampoules.

Table 1
Review of published DOC conditions of sampling, filtering and storage parameters since 1994 for different type of waters. Symbol "-" means that the information was not specified in the publication. PP for Vials refers to polypropylene, HDPE to high-density polyethylene, PTFE to polytetrafluoroethylene and PE to polyethylene. GF/F for Filtration refers to glass fiber filter, GF/D to glass microfiber filter, PVDF to polyvinylidene fluoride and PES to hydrophilic polyethersulfone. UP Water means ultra-pure water.

	Vials	Vial decontamination	Filtration	Bacterial inhibiting agent	Storage	Samples
Kaplan, 1994	Glass	550 °C, 6 h	0.7 µm GF/F or Gelman A/E glass microfiber or 0.45 µm Ag (Silas Flotronics) or 0.20 µM Anatops (alumina)	H_2SO_4 to pH 2 or 13.5 µM NaN_3 or 0.74 mM $HgCl_2$	5 °C	Freshwater, rainwater
Wiebinga and the Baar, 1998	PTFE	Acid washed (not detailed) + filled with UP water	0.2 µm polycarbonate	3 drops of 45% H_3PO_4 for 8 ml	5 °C	Seawater
Lara et al., 2001	PE	10% HCl	–	H_3PO_4	4 °C	Rainwater
Sharp et al., 2002	Glass	450 °C several hours (not precised)	0.7 µm GF/F	Ultra-clean H_3PO_4 1:1000	–	Seawater
Tue-Ngeun et al., 2005	–	3% Neutracon™, 12 h, 10% HCl, 12 h	0.7 µm GF/F	None	4 °C	Freshwater
Campos et al., 2007	Glass	Several, the best: 1 mmol L^{-1} Fe^{2+}, 100 mmol L^{-1} H_2O_2; pH 2.5, 1 h	0.7 µm GF/F	None	4 °C	Rainwater
Nemery et al., 2013	–	–	0.7 µm GF/F	50 µL HCl 1 N for 20 ml	4 °C	River
Helton et al., 2015	Polycarbonate or glass	Acid washed (not detailed)	0.22 µm nylon	50 µL H_3PO_4 for 40 ml	Frozen*	River
Moyer et al., 2015, Raymond et al., 2001	Glass	> 550 °C	3.9 µm GF/*D* + 0.7 µm GF/F	25 µl HCl high purity for 4 ml	–	River, estuary
Martin et al., 2018	Glass	–	0.2 µm Anodisc® (alumina)	100 µL 25% H_3PO_4 for 30 ml or 100 µL 50% H_2SO_4	4 °C	River, coastal water
Zhao et al., 2018	Glass	–	0.7 µm GF/*F* + 0.22 µm cellulose	–	–	River
Hemingway et al., 2019	Polycarbonate, HDPE	1.2 M HCl, 1 week	0.45 µm glass fiber	None	Frozen	Glacier meltwaters, precipitations
Huntington et al., 2019	Glass	–	0.7 µm GF/F or 0.45 µm Supor® (PES)	–	4 °C or frozen	River
Liu et al., 2019	–	Acid washed + combustion (not detailed)	0.20 µm Nuclepore (polycarbonate)	–	4 °C	Estuary
Wen et al., 2019	HDPE	–	0.45 µm glass fiber	–	4 °C	River
Wang et al., 2019	–	–	0.45 µm nylon	Acidification (not detailed)	Frozen	River
Zhao et al., 2020	Glass	–	0.7 µm GF/*F* + 0.22 µm cellulose	–	–	River
Garcia Martin 2021	HDPE	Acid washed (not detailed)	0.45 µm cellulose acetate	–	–	Estuary
Guo et al., 2021	Glass	450 °C, 4 h	0.7 µm GF/F	–	Frozen	Estuary
Sun et al., 2021	Glass	–	0.7 µm GF/F	–	4 °C	Subsurface water
Rogers et al., 2021	HDPE	Acid washed (not detailed)	0.7 µm GF/F	–	Frozen	River
Takano et al., 2021	PE	0.1 M HCl, 24 h	0.45 µm PVDF	–	Frozen	River
Xiaoni et al., 2021	–	10% HCl	0.7 µm GF/F	–	Frozen	River
Nisha et al., 2022	Glass	–	0.7 µm GF/F	–	4 °C	River
Takaki et al., 2022	Glass	–	0.7 µm GF/F	–	4 °C	River
Xu et al., 2021	Glass	–	0.45 µm (membrane not specified)	20 µl $HgCl_2$	4 °C	Shallow groundwater
Xue et al., 2022	Glass	5% HCl, > 24 *h* + 450 °C, > 5 h	0.22 µm PTFE	–	4 °C	River

Without acidification, freezing is recommended to avoid a loss of DOC in peatland waters of which concentrations exceed 8 mg L^{-1} [35] and filtering should be performed before freezing for runoff waters (up to 76 mg L^{-1}) [36].

In seawaters, DOC concentrations are usually below 1 mg L^{-1}, and recent papers recommend sample freezing, acidified in glass sealed ampoules [37] or not acidified in 1 L glass [34] or in HDPE vials [37,38]. Over a period of 1 year, freezing or a 4 °C storage gave similar DOC concentrations in samples acidified in sealed glass ampoules [37]. On a 3-year period, freezing without acidification gave similar results to acidification at room temperature in 40 ml glass vials (not sealed ampoules) [38]. More precisely, Halewood et al. [38] suggest to sample and to store directly in 40 ml glass vials adaptable to autosampler to minimize handling. Both Fourrier et al. [37] and Halewood et al. [38] were the only ones to pay attention to the effect of the vial closure, which, regarding to their results, is a key part in DOC preservation. They observed that screwed Bakelite® caps with unglued PTFE-lined septa [37] and caps (material not specified) equipped with re-used (*i.e.* pierced) PTFE septa on one side [38] both induced an increase in DOC concentration, up to

Table 2
Review of DOC publications devoted to sample containers and preservation techniques for different type of waters. PP refers to polypropylene, HDPE to high-density polyethylene, PC to polycarbonate, PTFE to polytetrafluoroethylene, GF/F to glass fiber filter, and PES to hydrophilic polyethersulfone.

	Vials	Vial decontamination	Cap/ septum	Tests	Storage time	Results	Recommendations	Samples
Tupas et al., 1994	15 ml PP, 100 ml HDPE 2–10 ml Sealed glass ampoules	PP, HDPE: 10% HCl Glass: 500 °C, 12 h	Septum not used	(a) 4 °C + acid (H_3PO_4) (b) (−20 °C or 4 °C), no acid	up to 5 months	No significant difference between frozen *versus* acidified samples (on glass ampoules of 10 ml). Smallest volumes (2 ml glass ampoules) induces differences in DOC. Samples not acidified induces a loss of DOC	Use of >100 ml HDPE bottles frozen, no acidification. For smaller volume, 10 ml glass sealed ampoule + acidification	Seawater
Peacock et al., 2015	60 ml plastic Nalgene®	not specified	not specified	Freezing *versus* 4 °C no acid in both	2–1082 days	At 4 °C: mean 20% loss on DOC after ~ 3 years. Freezing: slight decrease/increase (< 5%)	Freezing	Peatland waters DOC > 8 mg L^{-1}
Walker et al., 2017	1 L glass	540 °C, 2h	acid-cleaned PTFE caps	(a) Freezing (b) Room T °C + acidification (H_3PO_4)	59–380 days	Systematic loss of DOC with (b). Difference of 2.2 +/- 0.2 µM after 380 days between frozen/acidified samples	Freezing	Seawater
Nachimuthu et al., 2020	not specified	not specified	not specified	Freezing *versus* 4 °C no acid in both no filtration before storage (only before analysis)	30–45 days	At 4 °C: loss on DOC after 1 week, up to 35% after 27 days	Filtering before freezing	Runoff waters DOC up to 76 mg L^{-1}
Fourrier et al., 2022	30–125 ml HDPE bottles 30–60 ml brown glass	HDPE : GEOTRACES cleaning procedure Glass: HCl 0.01 *M* + 4 h 400 °C	Bakelite cap, Teflon septa	(a) Room T °C, dark (b) 4 °C + Suprapur® HCl (c) Freezing	up to 1 year	Gain of DOC with (a) and (b) in glass due to the Bakelite cap, which is not airtight, and alteration of septa under acid vapors. Best results with HDPE frozen (−4.1% gap after 1 year).	Use of HDPE or glass sealed ampoules, acidification, 4 °C or freezing	Seawater
Halewood et al., 2022	Glass, HDPE, PC	HDPE, PC: 1 M HCl Glass: 450 °C, 4h	PTFE/Silicon septa cleaned in 1 M HCl	(a) Room T °C, acidification, glass (b) Freezing, glass/HDPE/PC	0–3 years	No difference between (a) and (b) after 1 month. In glass: DOC is stable after 3 years freezing or with acid at room T °C after 2 years. Pierced septa induce high DOC increase.	Use of 40 ml glass vials adaptable to autosampler. Freezing in HDPE or in PC or in glass, or acidification in glass at room T °C. Never use caps with pierced septa	Seawater

a 2.5- and 1.5- factor respectively. They both related DOC contamination from potential airborne DOC deposition. Even if the study of Fourrier et al., was conducted with one aliquot and the one of Halewood et al., was done with 2 samples (temperature and time of storage not specified), they raise the question of the recycling of lab consumables, which is highly relevant. Additionnally, both studies did not deal with the combination of PP screwed caps, pierced septa, sample acidification, 40 ml glass vials adaptable to autosampler and freezing. Thus, more results are needed to assess the influence of this combination and DOC contamination during storage.

In this paper, we explored several materials and protocols to determine which one provides the best combination to measure the DOC blanks, *i.e.* the lowest DOC deviation from the baseline. We considered time saving, when other geochemical parameters, such as major and trace metals, are sampled and coupled to DOC analyses. This study was conducted using several vials and filter materials including polypropylene (PP) vials and cellulose acetate (CA) membranes that are commonly used for major cations sampling. We aimed to address the following questions: What is the contribution of PP vials *versus* glass vials to DOC contamination? Does the DOC contribution vary according to the type of glass vials (amber *versus* opaque)? What is the optimal calcination time for glass vial decontamination? Can classical filters used for trace metals be used for DOC analyses? Which acid for bacterial inhibition results in the lowest DOC contamination? Finally, do chemical sample matrices and preservation technique using a combination of screwed caps, pierced septa, glass vials and sample acidification influence DOC concentrations with time and temperature?

Material and methods

Analytical conditions

In the present study, DOC analyses were performed using a TOC-VCSN analyzer from Shimadzu associated to an ASI-V autosampler. After one to three rinses of the analytical syringe with the sample, the sampled volume (150 µl) was degassed during 1′30'' under air after automatic addition of 1.5 vol% HCl to remove inorganic carbon and volatile organic carbon. The sample was then injected in a Pt-catalyst tube heated at 720 °C for conversion of DOC into gaseous CO_2, and the remaining carbon (C) was subsequently detected by non-dispersive infra-red (NDIR) absorption. For each sample, the method implied two analyses and the mean concentration was given when the relative standard deviation (RSD) was below 2%. For RSD higher than 2%, a third analysis was automatically run and the closest replicates were kept. External calibrations were achieved by diluting a DOC certified standard from Chemlab at 100 mg L^{-1}, made from potassium hydrogen phthalate (KHP), to reach the range of sample concentrations. Accuracy was calculated by measuring a diluted DOC solution from another certified standard from Sigma at 1000 mg L^{-1}, made from dextrose and glutamic acid, and recoveries (*i.e.,* the ratio between the measured value to the theoretical one) calculated on all measurements returned 102% ($N = 25$, RSD = 7%). Precision (as RSD) was evaluated with the repeatability of several measurements of a same Chemlab standard during each sequence and was found to be better than (or equal to) 5%.

The ultra-pure (UP) water used was produced from drinking water that followed sequential purification methods to reach a final resistivity of 18.2 MΩ.cm and low total organic carbon certified below 10 µg L^{-1} by the manufacturer. The process of purification included water exposition to an intense multi- wavelength UV-radiation to provide continuous bacterial control and photo-oxidation of organic molecules. At the end, water passes through an ion-exchange cartridge to remove ionic impurities and through an ultimate polyethersulfone 0.2 µm bacterial filter. The DOC signal with a direct injection of UP water, *i.e.* without using autosampler and glass vials, is referred to 'machine blank' hereafter. This value was systematically removed from all DOC results when expressed in mg L^{-1}. When DOC values were too low to be accurately quantified using absolute units (mg L^{-1}), the results were presented as a deviation from the machine blank signal and were expressed as R_{SB}, *i.e.* a ratio of the raw sample signal to the one of the machine blank.

All glass vials used for this study were brown glass vials, either opaque or amber, adaptable onto the autosampler and equipped with screwed polypropylene caps containing PTFE septa on one side. Samples were analyzed using the autosampler. The caps and septa were washed 3 times with UP water before each use. All glass vials were cleaned and re-used between runs and, except mentioned hereafter, they were decontaminated within the day before use by rinsing one time with UP water and pyrolyzing at 450 °C during 3 h. At this temperature, residue of dissolved organic matter should be completely calcinated [39]. GF/F filters were decontaminated with this same protocol, the applied temperature being below the critical one for these filters (550 °C). Quantitative DOC results are expressed in mg L^{-1} of the detected remaining C and other elements are expressed in mol L^{-1} units.

Before sampling: choice of vial materials, filter/membranes and glass decontamination

Sample blanks using UP water were prepared in duplicates, and stored either in brown glass vials or in new PP vials, as the latter is commonly used for major cations sampling. Glass vials were quickly decontaminated at 500 °C during 1 h whereas PP vials did not undergo any decontamination. Polypropylene syringes were rinsed 3 times with 20 ml of UP water and filters were rinsed with at least 20 ml of UP water. In order to check if there was a significant and systematic DOC release from the material itself, three different membranes, *i.e.* cellulose acetate (CA), hydrophilic polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF, Sterivex®), and GF/F (Whathman®) filters were used to filtrate 30 ml of UP water. Membranes and filters were all 25 mm in diameter. The membranes, commonly called 'syringe filters', were directly adaptable on the syringe outlet, each one inserted inside a closed PP housing that does not allow the membrane to be dried before use. The GF/F filters were only discs and were used with an independent PP Swinnex® filter holder. GF/F filters and the associated glass syringe were decontaminated (500 °C, 1 h) and the PP Swinnex filter holder was immersed two days in a 1 N (3 vol%) HCl bath and then rinsed with UP water. After filtration, one set of sample blanks was acidified and the other one was not acidified. Acidification was done with analytical grade (p.a) 37 vol% HCl (0.5 ml in 30 ml).

Amber and opaque glass vials were distinguished with the GF/F filter test to see if any difference could be detected and come from the type of glass vials only. Samples were kept at 4 °C in the dark, and the DOC analyses were run within less than 24 h for glass vials and 48 h for PP vials (the difference of time being due to the duration of analytical sequence). Samples prepared in PP vials were transferred just before the analysis in decontaminated glass vials (500 °C, 1 h) for autosampling.

To test the protocol for glass decontamination, six series of ten glass vials were pre-contaminated overnight in 0.01 M citric acid [24] and then rinsed one time with UP water. One series did not undergo calcination whereas three series were calcinated at 500 °C for 1 h, 3 h, and 6 h. Two additional series were calcinated at 450 °C for 3 h and for 12 h to check any effect with decreasing temperature or increasing time. After calcination, 35 ml of UP water was filtered through 0.45 µm CA membrane pre-rinsed with 20 ml of UP water. Membranes were changed for each glass vial and the same PP syringe was kept for all vials. DOC concentration was then measured within the day after preparation.

On-site sampling: filter/membranes decontamination and sample acidification

The DOC contamination of four membranes (CA, hydrophilic PTFE, Sterivex® PVDF, Sterivex® polyethersulfone (PES)) and from GF/F filters was evaluated after filtration of 20 ml of UP water for each of them. This filtration step was repeated successively in 10 glass vials with the same filter and PP syringe, to observe the effect of filter washing. GF/F filter and glass vials were previously decontaminated for 6 h at 450 °C. Then, filter, membranes and syringes were not rinsed and PP syringe was changed with each filter and membrane. No acid was added and DOC analyses were performed immediately after preparation.

Acids were used as bacterial inhibiting agents instead of NaN_3 or $HgCl_2$ due to the high toxicity of these later chemicals and because $HgCl_2$ is not suitable with a Pt-catalyst [24]. DOC release was determined for hydrochloric acid of p.a. and Optima® grades, and for p.a. grade H_3PO_4. Indeed, even if Optima® grade HCl is certified for trace metal concentrations, nothing is specified concerning DOC. A volume of 20 ml of UP water was filtrated through hydrophilic 0.45 µm PTFE into glass vials and acidified either with 0.5 ml of 0.3 vol% HCl [5] for both HCl grades or with 670 µl of 2.5 vol% H_3PO_4 [7]. Polypropylene syringes and membranes were pre-rinsed 3 times with 20 ml of UP water before use and were changed for each acid. Brown glass vials were previously decontaminated during 6 h at 450 °C. Solutions of 0.3 vol% HCl and 2.5 vol% H_3PO_4 were prepared and stored into brown glass vials. Five replicates were prepared for each acid and DOC analyses were performed within 12 h.

Influence of ΣH_2S, Na, Ca matrices, effects of the combination of screwed caps, pierced septa, glass vials and acidification on DOC with time and conservation (4 °C or freezing)

To test the effects of sulfide ($\Sigma H_2S_{(aq)}$), sodium (Na^+) and calcium (Ca^{2+}) on DOC analyses, 1 mg L^{-1} DOC solutions were prepared from the 100 mg L^{-1} Chemlab standard diluted in different aqueous matrices, *i.e.* UP water, HCl acidified water, sulfide ($\Sigma H_2S_{(aq)}$), sodium bromide (NaBr), sodium chloride (NaCl) or calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$). The concentration of Na^+ and Ca^{2+} in the samples was 0.13 mol L^{-1} and 0.075 mol L^{-1} respectively (3 g L^{-1} for both). The solution of ΣH_2S standard was prepared following Tisserand et al. [28], then diluted to obtain a final sample concentration of 20 µmol L^{-1}. Such a ΣH_2S concentrations is typical of anoxic zone in stratified lakes [14]. Sample blanks without any DOC were also prepared using the different matrix solutions. All solutions were prepared in duplicates, in 40 ml glass vials with PP screwed caps equipped with re-used (*i.e.* pierced) PTFE septa on one side and DOC quantitative analyses began the day of preparation. After the first analysis, samples were bagged and a first set of samples was kept at 4 °C whereas a second set was stored at −18 °C. We made sure that the vial volume was enough to accommodate the sample expansion due to freezing. Then, samples were analyzed again after 30 to 45 days of storage.

To better monitor DOC evolution through time at low concentrations with similar caps, pierced septa and glass vials, a natural river water (*i.e.* Isère River, sampled near ISTerre laboratory in Saint Martin d'Hères, France), with DOC levels ranging from 0.9 to 2.6 mg L^{-1} at the time of analysis [5], was sampled and filled to the brim in a new HDPE 500 ml flask, pre-rinsed 3 times with the river water. Just after sampling and at the laboratory, a volume of 35 ml of sample was filtered through hydrophilic 0.45 µm PTFE membranes that were previously rinsed with 20 ml of sample. Eight aliquots of the sample were prepared in glass vials equipped with PP screwed caps and the pierced septa, divided into four replicates to be stored at 4 °C, and the four others at −18 °C. The same PP syringe was used to prepare the eight replicates whereas PTFE membrane was changed for each sample. Other replicates were prepared following the same protocol for a set of eight sample blanks (*i.e.* made with UP water). Another set of a 1 mg L^{-1} DOC solution was prepared, without filtration, from the 100 mg L^{-1} Chemlab standard. All samples were acidified following [5], *i.e.* by adding 0.88 ml of 0.3 vol% HCl for the 35 ml. DOC analyses at $t = 0$ were done within the day of preparation. Then, all samples were bagged and stored vertically to avoid any contact with the septum. Because of a limited volume of sample available in each vial, two alternative replicates, stored at 4 °C, were analyzed every 15 days and then put back in the fridge. Finally, after 2 months storage, frozen samples were thawed at ambient temperature during less than one day and analyzed in the wake with all other samples kept at 4 °C.

Method validation

Before sampling: choice of vial materials, filter/membranes and glass decontamination

The DOC values of blank samples using UP water were systematically higher in PP vials compared to glass vials (Fig. 1). The overshoots of mean blank to machine blank DOC concentration, referred to as the R_{SB} ratio, were in the same order of magnitude

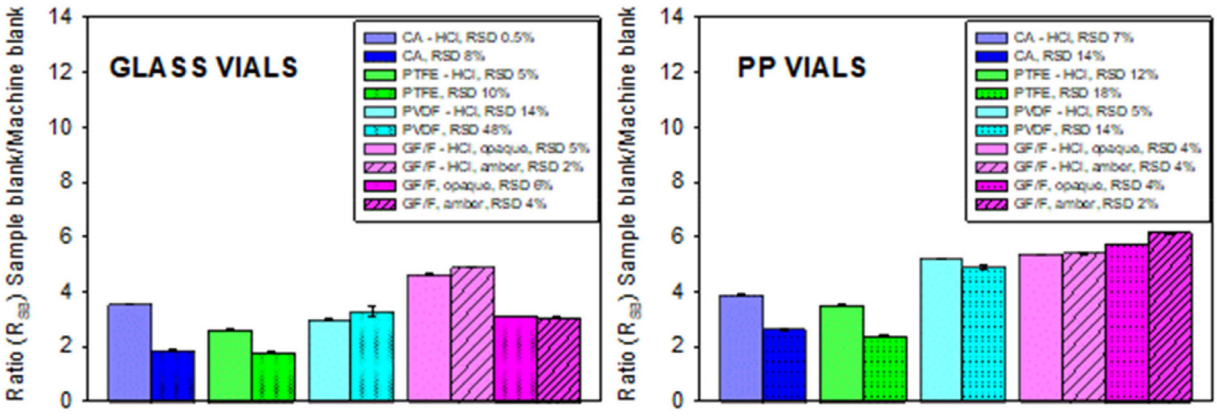

Fig. 1. Ratio (R_{SB}) of the mean of two sample blanks to machine blank DOC concentrations. Sample blank stands for filtered UP water stored with or without HCl, either in glass vials or polypropylene (PP) vials. The filters were Cellulose Acetate (CA), hydrophilic Polytetrafluoroethylene (PTFE), glass fiber filters (GF/F) and Polyvinylidene fluoride (PVDF). RSD values calculated on the duplicates are reported in the figure caption.

for glass vials, and ranged from 1.81 to 3.54 for the three 0.45 µm membranes whereas for PP vials, R_{SB} were higher with values ranging from 2.39 to 5.20. With GF/F filters, R_{SB} ranged from 3.07 to 4.88 in glass vials and from 5.36 to 6.14 in PP vials. In the case of opaque *versus* amber glass vials used with GF/F, no significant difference was obtained between both types, with recoveries on R_{SB} (values in amber *versus* opaque glass) ranging from 98 to 105% with the exclusive glass vial set and from 101 to 107% with storage in PP vial and then transfer into the distinguished glass vials for the analysis. In glass vials, a contribution from HCl to DOC concentration was observed, with recoveries on R_{SB} (values with HCl *versus* no HCl) ranging from 149 - 159% with GF/F to 191% with CA. Surprisingly, a small deficit with PVDF was observed (90%) with HCl addition. With PP vials, the contribution from HCl seemed to vary more randomly, representing either a deficit (R_{SB} recoveries at 88 - 94%, with GF/F) or an excess (R_{SB} recovery at 147%, with CA). This highlights that this DOC contamination or depletion does not come from HCl itself but rather from interactions between the flask material and HCl.

In glass vials and without HCl, the highest R_{SB} ranged from 3.07 (GF/F) to 3.30 (PVDF), whereas the contributions of the two 0.45 µm CA and PTFE membranes to DOC were the lowest and almost equivalent, at $R_{SB} = 1.85$ (CA) and $R_{SB} = 1.81$ (PTFE). However, even if calculated on two R_{SB} replicates, 0.45 µm PTFE membrane gave the two lowest R_{SB} without and with HCl at 1.81 and 2.62 respectively.

According to these results, glass vials, even with a fast decontamination (1 h), clearly represent the best material to minimize DOC contamination and, combined to these vials, 0.45 µm PTFE membranes seem to represent the most commendable filters.

The R_{SB} calculated from the mean DOC values of ten replicates on calcinated glass vials were found to be on the same order of magnitude at 2.1 (RSD = 21%) and 1.7 (RSD = 23%) for calcination at 500 °C lasting 1 h and 6 h, respectively (Fig. 2). Decreasing temperature to 450 °C with or without increasing calcination time still maintained R_{SB} in the same order of magnitude both for a 3 h-calcination ($R_{SB} = 2.3$, RSD = 17%) and for a 12 h-calcination ($R_{SB} = 2.4$, RSD = 12%). Although a 3 h calcination step gave the lowest value ($R_{SB} = 1.3$) at 500 °C, it also corresponded to the highest RSD (54%). Considering the errors bars on results (Fig. 2), they show no real difference in R_{SB} between the different calcination times (1 h to 12 h) or temperature (450 °C or 500 °C). Surprisingly, R_{SB}, obtained with vials rinsed one time with UP water but with no calcination, was low (1.6), but skipping the calcination step is not recommended, as the risk of contamination when re-using vials that have previously contained high DOC samples remains high.

On-site sampling: filter/membrane decontamination and sample acidification

For the five membranes and filter (0.45 µm CA, 0.45 µm hydrophilic PTFE, 0.45 µm PVDF, 0.7 µm GF/F and 0.22 µm PES), the filtration of the first 20 ml UP water resulted in a significantly higher R_{SB} compared to the other successive 20 ml filtration steps (Fig. 3). It should be noted that Sterivex® (PVDF or PES) were easy to use because of their large filtration surfaces and GF/F filters were easily torn if the filtration flow rate was too fast.

The use of PVDF resulted in the highest initial R_{SB} ratio (12.7), against 7.2, 6.1, 4.6 and 3.9 for CA, PTFE, GF/F, and PES respectively. The two following PVDF filtrates yielded $R_{SB} = 6.8$ and 4.5, followed by an average value at 3.5 for the seven other filtrates (RSD = 9%). For the other filters, the nine following filtrates returned R_{SB} values of 3.3 (RSD = 14%), 2.5 (RSD = 14%) and 1.8 (RSD = 22%) for CA, GF/F and PES respectively. The best RSD (5%), combined to a low R_{SB} (2.6), was obtained with PTFE filters.

These results show that the filters contribute to DOC signal. This DOC contribution from the filter was observed by Petitjean et al. [40], who used 0.45 µm membranes (type of membranes not specified) and found DOC concentration ranging from 3.5 mg L^{-1} after a 5 ml rinse and still at 0.48 mg L^{-1} after a 10 ml rinse. Rinsing volume is thus important and must be applied using either UP water or the sample itself, for sample blanks and sample respectively, with similar volumes of rinsing for blanks and samples. For GF/F filters, this rinsing must be done, for both sample blanks and samples, only with UP water and not with the sample, because these

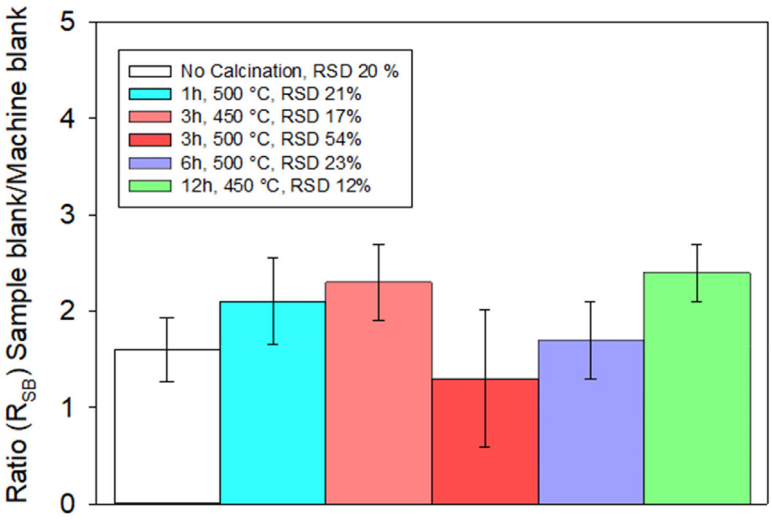

Fig. 2. Ratio (R_{SB}) of the mean of ten sample blanks to machine blank DOC values. Sample blank stands for filtered UP water after decontamination of glass vials either with only one UP water rinse, or coupled with a calcination at 500 °C from 1 h to 6 h, or at 450 °C during 3 h or 12 h. Relative Standard Deviation (RSD) are calculated on the 10 replicates.

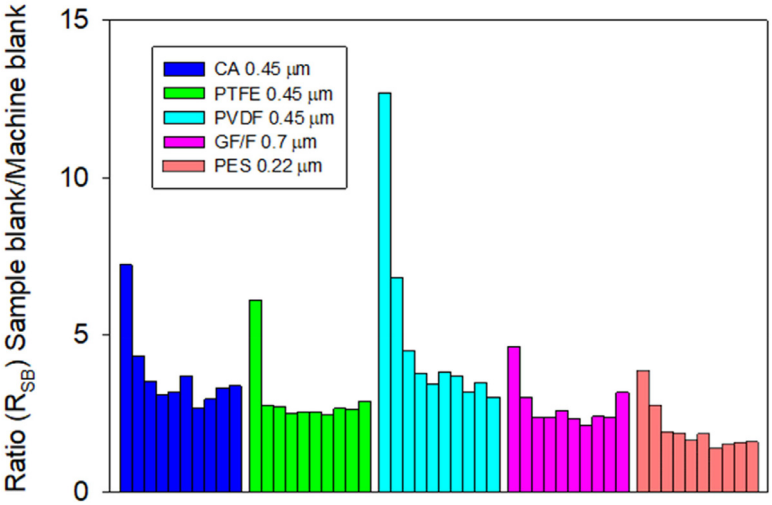

Fig. 3. Ratio (R_{SB}) of sample blanks to machine blank DOC values. Sample blank stands for 20 ml of filtered UP water through cellulose acetate (CA), polytetrafluoroethylene (PTFE), Sterivex® polyvinylidene fluoride (PVDF) membranes, glass fiber filters (GF/F) and Sterivex® polyethersulfone (PES) membranes for 10 successive filtrations using 20 ml of solution for each filtration step.

filters may be analyzed after sampling for the determination of particulate organic carbon, which could be reported to the filtered volume of sample.

Compared to Fig. 3 for the seven replicates with PTFE after three rinses of 20 ml filtration (mean R_{SB} = 2.6, RSD = 5%), acid addition increased DOC concentrations, such that R_{SB} reached values between 4.6 and 6.9 (Fig. 4). Analytical (p.a.) or Optima® grade HCl returned similar DOC contributions with a mean R_{SB} value for the five replicates at 5.0 and 4.9 (RSD = 5% and 10% respectively) (Fig. 4). Results obtained with H_3PO_4 were slightly higher with a mean R_{SB} at 6.1 (RSD = 8%). Thus, both HCl grades seem to be the best to acidify samples and the Optima® HCI, used for acidification of natural waters for trace metals such as dissolved mercury [14], can also be used for DOC measurements. Even if acidification is commonly done under H_3PO_4 in the literature (Table 1), HCl remains a better choice to preserve in the long term the Pt catalytic tube of the analyzer (C. Consolino, pers. comm.). Sample blanks prepared with UP water should thus be acidified with the same concentration as the samples.

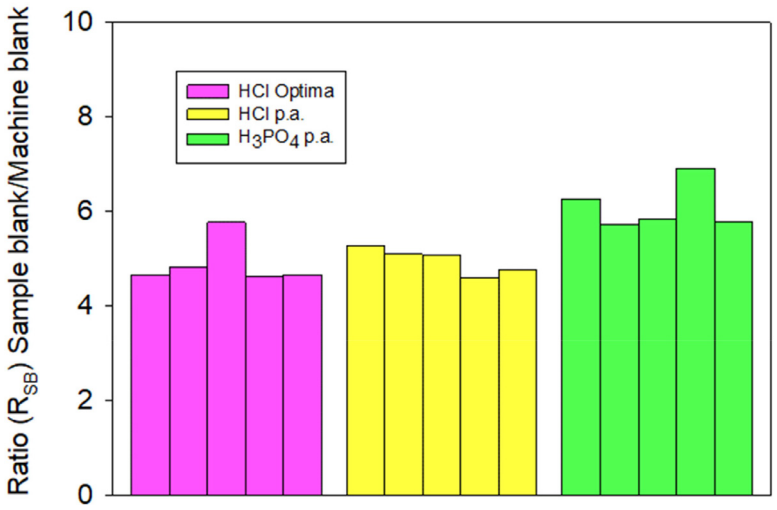

Fig. 4. Ratio (R_{SB}) of sample blanks to machine blank DOC values. Sample blank stands for 20 ml of filtered UP water through hydrophilic PTFE filter membranes and acidified with Optima® grade HCl, analytical grade (p.a) HCl (0.5 ml of 0.3 vol% HCl [5]) or analytical grade (p.a.) H_3PO_4 (670 µl of 2.5 vol% H_3PO_4 [7]). The five bars for each acid represent the five repetitions of the preparation in each acid.

Table 3
Raw (i.e. without sample blank subtracted to samples) DOC concentrations (mg L^{-1}) at $t = 0$ and after 1 to 1.5 month for UP water and 1 mg L^{-1} DOC, stored at 4 °C or −18 °C, in the presence of HCl (0.5 ml of 0.3 vol% HCl per 20 ml [5]) and/or 20 µmol L^{-1} ΣH_2S, or with (*) HCl diluted 10 times and sodium bromide (NaBr), or sodium chloride (NaCl) or calcium nitrate $Ca(NO_3)_2{\cdot}4H_2O$, with [Na]= 0.13 mol L^{-1} and [Ca]= 0.075 mol L^{-1}. Sample blank signal with UP water were subtracted to corresponding DOC sample only for the calculation of Recovery %.

	DOC (mg L^{-1}) $t = 0$	Revovery% measured/theorical value $t = 0$	DOC (mg L^{-1}) $1 < t < 1.5$ month	$[DOC]_t$ / $[DOC]_{t=0}$ $1 < t < 1.5$ month
Storage 4 °C				
UP Water	0.13		0.22	1.7
UP Water + HCl	0.21		0.36	1.7
UP Water + HCl + ΣH_2S	0.83		0.98	1.2
DOC 0.96 mg L^{-1}	1.07	98	1.14	1.1
DOC 0.97 mg L^{-1} + HCl	1.11	92	1.65	1.5
DOC 0.96 mg L^{-1} + HCl + ΣH_2S	1.70	91	1.74	1.0
Storage −18 °C				
UP Water	0.12		17.24	142
UP Water + HCl	0.20		11.98	59
UP Water + HCl + ΣH_2S	0.71		12.58	18
DOC 1.13 mg L^{-1}	1.25	100	26.59	21
DOC 1.01 mg L^{-1} + HCl	1.16	94	11.62	10
DOC 0.98 mg L^{-1} + HCl + ΣH_2S	1.67	98	10.32	6
UP Water + HCl* + NaBr	0.20		52.64	267
DOC 1.03 mg L^{-1} + HCl* + NaBr	1.16	93	25.17	22
DOC 1.06 mg L^{-1} + HCl* + NaBr	1.15	90	30.82	27
UP Water + HCl* + NaCl	0.34		60.96	180
DOC 1.02 mg L^{-1} + HCl* + NaCl	1.27	92	37.66	30
DOC 1.06 mg L^{-1} + HCl* + NaCl	1.31	91	36.02	28
UP Water + HCl* + $Ca(NO_3)_2$	0.13		12.91	96
DOC 1.02 mg L^{-1} + HCl* + $Ca(NO_3)_2$	1.07	92	17.27	16
DOC 1.01 mg L^{-1} + HCl* + $Ca(NO_3)_2$	1.09	95	6.95	6

Influence of ΣH_2S, Na, Ca matrices, effects of the combination of screwed caps, pierced septa, glass vials and acidification on DOC with time and conservation (4 °C or freezing)

Duplicates of the 1 mg L^{-1} DOC Chemlab solution were prepared with different matrices and were analyzed after two different time durations. At $t = 0$, recoveries (*i.e.*, the ratio of measured DOC concentration to the theoretical one) were less than 10% deviation from 100% and ranged between 98 - 100% without HCl, 92 - 94% with HCl and 91 - 98% with HCl+ΣH_2S (Table 3). The presence of ΣH_2S did not influence DOC concentrations. Similarly, the presence of Na and Ca in the two 1 mg L^{-1} DOC solutions slightly acidified with HCl resulted in recoveries at 90 – 93%, 91 – 92% and 92 - 95% in NaBr, NaCl and $Ca(NO_3)_2$ matrices respectively. Thus, the impact of salts and sulfur on combustion efficiency during DOC analyses is relatively low, less or equal to 10%.

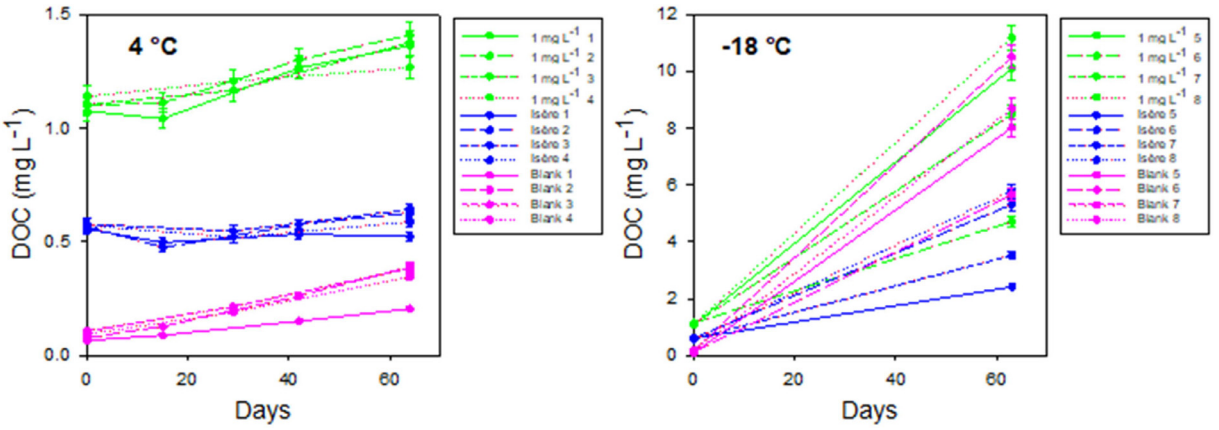

Fig. 5. Monitoring of raw concentrations (i.e. without blank subtracted to samples) of Isère River sample, 1 mg L^{-1} DOC solution and blank of UP water stored in glass vials at 4 °C for 4 replicates (n°1 to 4) and at −18 °C for 4 other replicates (n°5 to 8).

After a one-month period at 4 °C, DOC concentrations in the 1 mg L^{-1} solutions were almost the same for samples prepared without HCl and with HCl+ΣH_2S, respectively. With HCl only, the measured DOC concentrations increased by a factor of 1.5.

When kept at −18 °C, concentrations increased dramatically for all 1 mg L^{-1} samples, ranging from a 6-time increase with HCl+ΣH_2S or HCl+$Ca(NO_3)_2$ to a 30-time increase with HCl+NaCl. These large increases, even observed in sample blanks that reached a concentration at 60.96 mg L^{-1} with HCl+NaCl, seem to fluctuate randomly. One possible explanation for this DOC contamination, much higher after freezing compared to 4 °C, is an intensive capture of airborne carbon through the vial closures, *i.e.* the PP screwed caps equipped with pierced septa, since vials caped with PP caps and unpierced septa had previously proven reliable for samples frozen over durations as long as 3 years [38].

To better assess DOC contamination through time under the same preservation conditions, a natural Isère River sample was analyzed following the same method as that applied to the samples reported in Table 3, *i.e.* without subtracting sample blank concentration (Fig. 5). Isère samples were found at concentrations close to 0.5 mg L^{-1}.

After 2 weeks storage at 4 °C, Isère DOC concentrations still remained at 84% to 89% of their value at $t = 0$ on two duplicates. The DOC concentrations were still at 91 - 95% of the $t = 0$ value for the other 2 duplicates after 29 days. After 2 months storage, recoveries ranged between 94 - 111% for the 4 replicates. This shows that samples were relatively stable.

The evolution of the 1 mg L^{-1} sample was slightly different. After 2 weeks at 4 °C, concentrations for duplicates were still stable with recoveries at 97 - 101% and still at 106% after 29 days for the two other duplicates. Beyond this time, concentration gradually increased to reach recoveries from 111% to 128% after 2 months. Importantly, sample blanks (with UP water) concentration was not negligible, even at $t = 0$ (mean DOC = 0.1 mg L^{-1}, RSD 21%), and kept increasing in the weeks later. Such an evolution has a major impact on the determination of low sample concentrations. While the concentration of the blanks was only semi quantitative at $t = 0$ because they were below the lowest calibration point at 0.2 mg L^{-1} as one could expect, a slight increase of 1.3 to 1.7 factor was already observed after 2 weeks. Then, increases in concentration were high and ranged from 0.2 to 0.4 mg L^{-1} after 2 months. Thus, when calculating the ratio of concentrations at t compared to $t = 0$, including the subtraction of the sample blank to samples, values were at 0.6 - 0.7 and 0.4 – 0.6 for Isère sample and at 0.9 and 0.8 - 1.1 for the 1 mg L^{-1} sample, after 15 and 64 days respectively.

Even if precaution was taken to avoid contamination of the septum with no contact with the solution, freezing the samples induced a dramatic increase in concentrations (Fig. 5). At $t = 0$, the analyses carried out on Isère sample gave a mean concentration of the four replicates at 0.6 mg L^{-1} (RSD = 4%). After two months at −18 °C, the concentrations increased and ranged from 2.4 to 5.8 mg L^{-1}. These increases are drastic but not homogeneous from one replicate to another (Table 3). Increases of 1 mg L^{-1} sample and UP water blank were even more than those of Isère sample, with factors up to 10 and 60, respectively.

As detailed above, and referring to Table 2, the use of sealed glass ampoules, acidification and freezing or 40 ml glass vials with acidification and room temperature or without acidification and freezing had previously been tested and are reliable preservation techniques for DOC samples at concentrations below 1 mg L^{-1}. Thus, the DOC contamination observed here can only be linked to a part of the combination involving the vial closure, *i.e.* screwed PP caps equipped with pierced septa. Tested on one aliquot, Fourrier et al., 2022 observed DOC increase (2.5-factor) after a year when a screwed Bakelite® cap with unglued PTFE septum was used at room temperature. On two samples, pierced septa induced increase of 1.5-factor (acidification, temperature and time of storage not specified) [38]. As the latter study recommended the use of 40 ml glass vials adaptable to autosampler and unpierced septa, they probably used screwed caps when they observed DOC stability after 3 years at −18 °C (without acid) or after 2 years at room temperature (with acid). Thus, here, the influence of pierced septa combined with sample acidification rather than the PP screwed caps or the 40 ml glass vials, are most likely the cause of the DOC contamination under freezing, even if further research would complete this hypothesis. With pierced septa, Isère and 1 mg L^{-1} samples were relatively stable within 2 weeks at 4 °C, but DOC increase in the sample blanks induced significant offsets on DOC sample after blank subtraction. When frozen and thawed after 2 months, a dramatic DOC contamination was evidenced, and suggest that freezing and thawing processes favours DOC contamination

even more. In addition, as freezing and thawing can cause a change in the nature of the DOC through a loss of aromatic substances [41], this way of storage should be employed according to required analyses.

Recommendations and good practices for DOC measurements ≤ 1 mg L^{-1}

The results of this study demonstrated that the most suitable vials to be used for DOC are brown glass vials, either amber or opaque. Before use, a rinse of the vials with UP water and calcination for at least 1 h at 450 °C or 500 °C ensures the removal of organic residues.

GF/F filters should be used when suspended particles have to be sampled in the same time as DOC. Before use, filters should be calcinated in the same way as the glass vials. If suspended particles are not sampled, the use of the same membranes to monitor either DOC or other geochemical parameters will allow to save time and, after analysis, comparisons of dissolved species below the same cut-off value. In that case, 0.45 µm hydrophilic PTFE can be used.

At the site of sampling and before use, filters and membranes must be systematically pre-rinsed with 20 ml of UP water. If no UP water is available on site, the sample itself can be used to rinse PTFE membranes if sample's turbidity is low enough not to induce a rapid clogging of the membrane that would impede the filtration. If sample's turbidity is high, the volume of rinsing should be adapted to the site conditions. In any cases, a unique volume of rinsing should be absolutely applied for filters and membranes at all sampling points to get comparable values of samples, with similar DOC contribution from the filters or membranes. Sample acidification can be done by using both Analytical (p.a) or Optima® grade HCl (0.5 ml of 0.3 vol% HCl per 20 ml of sample [5]). Sample blanks using UP water should be prepared in exactly the same conditions as for the samples, to subtract DOC concentration from the blank to the one of the sample. The use of pierced PTFE septa with sample acidification must be proscribed. External standards calibration points, prepared in UP water by diluting a certified DOC solution, will permit quantitative analysis. With standards prepared in UP water, the loss of signal will be below or equal to 10% in the case of sample matrix-rich in Na (0.13 mol L^{-1}), Ca (0.075 mol L^{-1}) or ΣH_2S (20 µmol L^{-1}). Accuracy and precision of the results for each analytical sequence can be evaluated by repeating analysis on two independent certified DOC standard solutions.

Conclusions

The effect on DOC contamination from glass and plastic vials, from filters and from acids were studied. Matrix effects, including ΣH_2S, Na, Ca, as well as the effect of pierced septa with PTFE side, temperature on storage (4 °C *versus* −18 °C) and the time of storage, were evaluated on quantitative DOC analysis. Especially, we focused on concentrations lower or equal to 1 mg L^{-1}, corresponding to low values that are more sensitive to contamination / degradation, and out of the range of the existing ISO protocol. The lowest DOC contaminations with the best precision was obtained with brown glass vials decontaminated at least 1 h at 450 °C or 500 °C, filtration through 0.45 µm hydrophilic PTFE membranes previously rinsed with 20 ml and an acidification with analytical (p.a) or Optima® grade HCl (0.5 ml of 0.3 vol% HCl for 20 ml of sample). Sulfides, sodium and calcium do not induce matrix effects higher than 10% loss on the analysis. The use of pierced septa with acidified samples stored at 4 °C resulted in a light increase in the DOC value after 2 months, while under freezing, this resulted in a dramatic in measured DOC concentration. In that respect, recycling of vial closures of pierced septa with PTFE side used with acidified samples must be prohibited for storage at both 4 °C and −18 °C.

Ethics statements

There is no relevant statement listed in the Method article template that corresponds to this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Delphine Tisserand: Investigation, Conceptualization, Validation, Writing – review & editing. **Damien Daval:** Conceptualization, Validation. **Laurent Truche:** Conceptualization, Validation. **Alejandro Fernandez-Martinez:** Conceptualization, Validation. **Géraldine Sarret:** Conceptualization, Validation. **Lorenzo Spadini:** Conceptualization, Validation. **Julien Némery:** Conceptualization, Validation.

Data availability

Data will be made available on request.

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